TOXICOLOGY PROGRAM
eport Series

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TITANOCENE DICHLORIDE

(CAS NO. 1271-19-8)

IN F344/N RATS

(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF TITANOCENE DICHLORIDE

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IN F344/N RATS

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NATIONAL TOXICOLOGY PROGRAM
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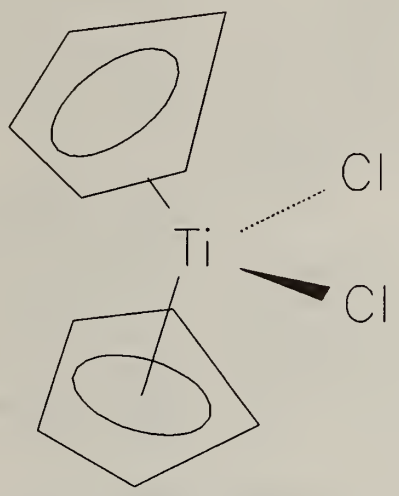
CONTENTS

ABSTRACT



TITANOCENE DICHLORIDE

CAS No. 1271-19-8

Chemical Formula: $(C_5H_5)_2TiCl_2$ Molecular Weight: 248.99

Synonyms: Titanium ferrocene; biscyclopentadienyltitanium dichloride; dichlorodi-π-cyclopentadienyltitanium; dichlorobis (η^5-2,4-cyclopentadien-1-yl)titanium; dicyclopentadienyltitanium dichloride; dichlorodicyclopentadienyltitanium; dichlorotitanocene; dicyclopentadienyldichlorotitanium; dichlorobis(π-cyclopentadienyl)titanium; bis(η^5-cyclopentadienyl)titanium dichloride; dichlorobis(η^5-cyclopentadienyl)titanium; dichlorobiscyclopentadienyl titanium; dichlorobis(1,3-cyclopentadiene)titanium; bis(cyclopentadienyl)dichlorotitanium

Titanocene dichloride is an organometallic compound composed of two cyclopentadienyl rings, titanium, and chloride. It is used as a cocatalyst in polymerization reactions. Toxicology and carcinogenesis studies were conducted by administering titanocene dichloride (greater than 98% pure) in corn oil by gavage to groups of F344/N rats for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in Chinese hamster ovary cells.

14-Day and 13-Week Studies
In the 14-day studies, titanocene dichloride was administered at doses of 0, 65, 125, 250, 500, or 1,000 mg/kg. All high-dose rats and four of the five male and two of the five female rats given 500 mg/kg died during the studies. A dose-related decrease in body weight gain was seen in rats given 125, 250, 500, and 1,000 mg/kg. Lesions related to chemical administration included hepatocellular necrosis, tubule necrosis in the kidney, erosions and ulcers of the glandular stomach, and hyperplasia of the forestomach epithelium.

The 13-week studies were conducted by administering titanocene dichloride at doses of 0, 8, 16, 31, 62, or 125 mg/kg. One female rat in the 125 mg/kg dose group died from chemical toxicity during the fourth week of the studies. Body weight gain was lower in rats given 62 or 125 mg/kg than in control

groups. Treatment-associated histopathologic lesions were seen in the stomachs of high-dose males and all groups of females given titanocene dichloride. These lesions included hyperplasia and metaplasia of the glandular stomach and hyperplasia and hyperkeratosis of the forestomach.

Body Weight and Survival in the 2-Year Studies
The doses selected for the 2-year studies in rats (0, 25, and 50 mg/kg) were based on the potentially life-threatening nature of the glandular stomach lesions and the decreased body weight gain compared to controls seen in the 62 and 125 mg/kg dose groups in the 13-week studies.

The final mean body weights of high-dose males and females were 91% and 89% of controls, respectively. The 2-year survival rates for males in the control, low-, and high-dose groups were 41/60, 30/60, and 24/60; survival rates for female rats were 37/60, 30/61, and 31/60.

Nonneoplastic and Neoplastic Effects in the 2-Year Studies
The principal toxic effects associated with the administration of titanocene dichloride for 2 years occurred in the stomach. The lesions in the stomach were seen at the 15-month interim evaluations and were similar to, but less severe than, those observed at 2 years. The lesions included focal erosions of the glandular mucosa with an associated inflammatory response, hyperplasia and metaplasia of the epithelium of the fundic glands, and fibrosis of the lamina propria and submucosa. Forestomach lesions included focal acanthosis (hyperplasia) and hyperkeratosis of the stratified squamous epithelium. Squamous cell papillomas of the forestomach were seen in four low-dose males, one high-dose male, one low-dose female, and two high-dose females; none were observed in controls. A squamous cell carcinoma of the forestomach occurred in one low-dose male and a benign basosquamous tumor occurred in one high-dose male.

Accumulations of macrophages with blue-gray pigment believed to contain titanium were present in many organs of dosed rats including the gastrointestinal tract, liver, lung, and lymph nodes. A dose-related increase in the incidence of inflammation of the nasal mucosa and lung also occurred and was attributed to reflux and/or regurgitation and aspiration of gavage solution due to the severe stomach lesions.

Genetic Toxicology
Titanocene dichloride was mutagenic in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation (S9); it was not mutagenic in TA100 with S9, nor was it mutagenic in TA1535, TA1537, or TA98 with or without S9. Titanocene dichloride did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells, with or without S9.

Conclusions
Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity*[*] of titanocene dichloride in male F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas, squamous cell carcinoma, and basosquamous tumor benign. There was *equivocal evidence of carcinogenic activity* of titanocene dichloride in female F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas.

Nonneoplastic lesions associated with the administration of titanocene dichloride for up to 2 years included erosions and inflammation of the gastric mucosa, hyperplasia and metaplasia of the fundic glands with fibrosis of the lamina propria in the glandular stomach, and acanthosis (hyperplasia) and hyperkeratosis of the forestomach epithelium.

[*] Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Titanocene Dichloride

Variable	Male F344/N Rats	Female F344/N Rats
Doses	0, 25, or 50 mg/kg in corn oil by gavage	0, 25, or 50 mg/kg in corn oil by gavage
Body weights	Dosed lower than controls	Dosed lower than controls
2-Year survival rates	41/60, 30/60, 24/60	37/60, 30/61, 31/60
Nonneoplastic effects	Glandular stomach: erosions - 1/58, 9/59, 13/58; inflammation - 0/58, 9/59, 10/58; hyperplasia - 0/58, 10/59, 24/58; metaplasia - 0/58, 26/59, 36/58; fibrosis - 0/58, 30/59, 37/58; fat proliferation - 0/59, 2/59, 14/60 Forestomach: acanthosis (epithelial hyperplasia) - 8/57, 25/59, 26/59; hyperkeratosis - 5/57, 13/59, 17/59 Liver: granulomatous inflammation - 0/60, 16/59, 14/60 Various organs: pigmentation	Glandular stomach: erosions - 2/60, 11/60, 10/60; inflammation - 0/60, 4/60, 5/60; hyperplasia - 0/60, 24/60, 23/60; metaplasia - 0/60, 36/60, 51/60; fibrosis - 0/60, 39/60, 51/60; fat proliferation - 0/60, 15/60, 41/60 Forestomach: acanthosis (epithelial hyperplasia) - 11/60, 20/60, 27/60; hyperkeratosis - 10/60, 23/60, 21/60 Liver: granulomatous inflammation - 6/60, 24/60, 33/60 Various organs: pigmentation
Neoplastic effects[a]	Forestomach: squamous cell papilloma - 0/60, 4/60, 1/60; squamous cell carcinoma - 0/60, 1/60, 0/60; basosquamous tumor benign - 0/60, 0/60, 1/60	Forestomach: squamous cell papilloma - 0/60, 1/61, 2/60
Level of evidence of carcinogenic activity	Equivocal evidence	Equivocal evidence
Genetic toxicology		
Salmonella typhimurium		
Gene mutation:	Positive without S9 in strain TA100; negative with and without S9 in strain TA1535, TA1537, or TA98	
Sister chromatid exchanges		
Chinese hamster ovary cells *in vitro*:	Negative with and without S9	
Chromosomal aberrations		
Chinese hamster ovary cells *in vitro*:	Negative with and without S9	

[a] Number with lesion/total evaluated

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the NTP draft Technical Report on titanocene dichloride on November 19, 1990 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS

On November 19, 1990, the draft Technical Report on the toxicology and carcinogenesis studies of titanocene dichloride received public review by the National Toxicology Program (NTP) Board of Scientific Counselors' Technical Reports Review Committee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of titanocene dichloride by discussing the uses, experimental design, survival and body weight, and compound-related nonneoplastic and neoplastic lesions in male and female rats. The proposed conclusion was *equivocal evidence of carcinogenic activity* of titanocene dichloride for male and female F344/N rats.

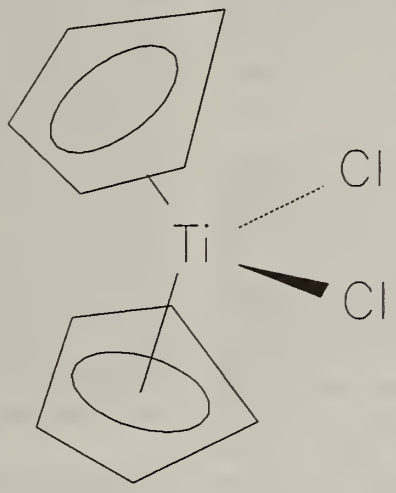
Dr. Gold, a principal reviewer, agreed with the conclusion in female rats but thought consideration should be given to changing the conclusion in male rats to *some evidence of carcinogenic activity.* The incidences of squamous cell papillomas of the forestomach were: controls, 0/60; low-dose, 4/60; high-dose, 1/60. Additionally, there was a carcinoma in the low-dose group. She based her proposed change on the rarity of these tumors, 0.3% in both male and female historical controls, with only one carcinoma in the data base, and supporting significant increases in forestomach hyperplasias in both low- and high-dose groups. Dr. Gold asked that the NTP consider reporting incidences for nonneoplastic lesions listed in the summary table in the Abstract.

Dr. Silbergeld, the second principal reviewer, agreed with the conclusions. She pointed out references in the discussion indicating more clearcut evidence of tumorigenesis from studies by inhalation or injection routes. She wondered if there was a potential for inhalation exposure in the workplace. Dr. J. Haartz, NIOSH, said that to her knowledge current use is only in research laboratories.

Dr. Hayden, the third principal reviewer, agreed with the conclusion for female rats but also thought consideration should be given to changing the conclusion for male rats to *some evidence of carcinogenic activity.* He based this on: (1) a statistically significant increase of gastric squamous cell papillomas that exceeded those found in study controls, historical controls at the study site, and NTP overall historical controls, (2) a lack of inference that stomach tumor incidence in study controls was below that expected for historical controls, and (3) a possibility that an increased incidence of gastric neoplasia would have been seen in high-dose males had more survived to term. Dr. S. Eustis, NIEHS, responded that there was not a dose response in male rats, the doses given were sufficient to cause considerable toxicity in the forestomach, the numbers of tumors were few, and perhaps most importantly, all but one of the papillomas (in both sexes) occurred at the limiting ridge, which is where all of the forestomach toxicity occurred. Further, the hyperplasias observed represented the kind that one sees as a regenerative response to toxicity rather than a preneoplastic lesion.

Dr. Hayden moved that the Technical Report on titanocene dichloride be accepted with the revisions discussed and the conclusions as written for male and female rats, *equivocal evidence of carcinogenic activity.* Dr. Goodman seconded the motion, which was accepted unanimously with eleven votes (Dr. Silbergeld absent).

INTRODUCTION



TITANOCENE DICHLORIDE

CAS No. 1271-19-8

Chemical Formula: $(C_5H_5)_2TiCl_2$ Molecular Weight: 248.99

Synonyms: Titanium ferrocene;biscyclopentadienyltitanium dichloride; dichlorodi-π-cyclopentadienyltitanium; dichlorobis(η^5-2,4-cyclopentadien-1-yl)titanium; dicyclopentadienyltitanium dichloride; dichlorodicyclopentadienyltitanium; dichlorotitanocene; dicyclopentadienyldichlorotitanium; dichlorobis(π-cyclopentadienyl)titanium; bis(η^5-cyclopentadienyl)titanium dichloride; dichlorobis(η^5-cyclopentadienyl)titanium; dichlorobiscyclopentadienyl titanium; dichlorobis(1,3-cyclopentadiene)titanium; bis(cyclopentadienyl)dichlorotitanium

Physical and Chemical Properties, Use, and Exposure

Titanocene dichloride is an organometallic compound composed of two cyclopentadienyl rings, titanium, and chloride (Clearfield *et al.*, 1975). It is moderately soluble in toluene, chloroform, and alcohol and is sparingly soluble in water, petroleum ether, benzene, ether, carbon disulfide, and carbon tetrachloride (*The Merck Index*, 1983). Titanocene dichloride forms bright red crystals when crystallized from toluene solutions. Titanocene dichloride has limited use as a cocatalyst for polymerization reactions (Hawley, 1977; Fieser *et al.*, 1984). The synthesis of titanocene dichloride was first reported in 1954 (Wilkinson and Birmingham, 1954). This compound is produced in limited quantities in the United States. No information was available on human exposure to this compound in the workplace.

Metabolism and Distribution

Following a single intraperitoneal injection of 60 mg/kg titanocene dichloride to NMRI mice, 10% of the total dose administered (measured as titanium by atomic absorption spectroscopy) was present in the liver at 24 and 48 hours. Titanium was also found in the intestine, kidney, lung, blood, and muscle, but not in the brain (Köpf-Maier *et al.*, 1988). Titanocene dichloride was administered intraperitoneally at a dose of 60 or 80 mg/kg to CF_1 mice. By electron spectroscopic imaging tech-

niques, titanium was found localized in the cytoplasm of the Kupffer cells lining the hepatic sinusoids (Köpf-Maier and Martin, 1989).

Toxicity and Biological Properties

The LD_{50} values reported for titanocene dichloride administered intraperitoneally were 25 mg/kg in rats and 60 mg/kg in mice (NCI, 1964). The LD_{50} for intravenous administration in mice was 180 mg/kg (NIOSH, 1981).

Titanocene dichloride was investigated for antineoplastic potential and was found to have antitumor activity (Köpf and Köpf-Maier, 1979; Köpf-Maier *et al.*, 1980a; Köpf-Maier and Köpf, 1986a). *Cis*-platinum (*cis*-diamminedichloroplatinum), a known antitumor agent, usually administered intravenously, is also a metal-containing compound which enters cells by diffusion, and once inside the cell, the chloride atoms are hydrolyzed, forming the activated drug species (Gilman *et al.*, 1985). Both of these antitumor agents use a "carrier" ligand (in the case of titanocene dichloride the carrier is cyclopentadienyl, C_5H_5) to transfer the metal moiety to the site of action in the diseased tissue. The halide groups represent the dissociable adjacent ligands, which by their replacement allow the active moiety to interact with the site of action, which is probably nucleic acids (Köpf-Maier *et al.*, 1980b).

A single intraperitoneal injection of 30 to 60 mg/kg titanocene dichloride increased the survival of CF_1 mice injected with Ehrlich ascites tumor cells (Köpf-Maier *et al.*, 1980a,b) and of DBA/2 mice injected with lymphoid leukemia L1210 or lymphocytic leukemia P388 (Köpf-Maier *et al.*, 1981). Titanocene dichloride also inhibited the growth of a human colon adenocarcinoma transplanted to athymic mice (Köpf-Maier *et al.*, 1985).

Examination of ascitic fluid from mice implanted with Ehrlich ascites tumor cells and subsequently treated with titanocene dichloride by intraperitoneal injection revealed that treatment with this compound induced mitotic aberrations in the tumor cells (Köpf-Maier, 1982). In studies of Ehrlich ascites tumor cells cultured *in vitro*, titanocene dichloride treatment caused an accumulation of cells in the late S phase and in the G_2 phase of the cell cycle (Köpf-Maier *et al.*, 1983).

The antitumor activities of titanocene dichloride have been demonstrated in a variety of other tumor/rodent model systems (Köpf-Maier and Gerlach, 1986a,b; Köpf-Maier and Köpf, 1986b; 1987; Köpf-Maier, 1987; 1988; 1989).

Reproductive Toxicity

The teratogenic and embryotoxic effects of titanocene dichloride have been studied in NMRI mice. Single doses of titanocene dichloride (30 or 60 mg/kg) were administered intraperitoneally to pregnant mice on days 8, 10, 12, 14, or 16 of gestation, and the fetuses were removed on day 18 and were examined for malformations. Treatment with titanocene dichloride was associated with an increase in the incidence of cleft palate and costal malformations and a reduction in the number of live fetuses per litter (Köpf-Maier and Erkenswick, 1984). Titanocene dichloride has been found to increase serum levels of cortisol in pregnant as well as in nonpregnant animals (Köpf-Maier, 1985).

Carcinogenicity

In Fischer 344/N rats receiving 25 injections of 8 mg titanocene dichloride in the right thigh muscle, 3 of 25 females and 2 of 25 males developed fibrosarcomas at the injection site. In addition, some of the treated animals developed hepatomas and malignant lymphomas of the spleen. Details of the study were not reported (Furst and Haro, 1969a,b; Furst and Schlauder, 1971; WHO, 1982). It has also been reported that titanium metal can cause fibrosarcomas at the site of local injection in rats (Furst, 1971; WHO, 1982). Titanium potassium oxalate administered in drinking water to Swiss mice (Charles River) at a concentration of 5 mg/L from weaning to natural death had no carcinogenic effects related to chemical administration (Schroeder *et al.*, 1964; Schroeder and Mitchener, 1975). Titanocene dichloride was positive in *in vitro* transformation tests in Balb/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344/N rat embryo cells (Dunkel *et al.*, 1981).

Genetic Toxicology

The limited mutagenicity data available for titanocene dichloride indicate that the chemical is mutagenic *in vitro* (induction of DNA damage in mammalian cells and gene mutations in *Salmonella typhimurium*), but has no clastogenic activity as

measured by the induction of sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells (Appendix C, Tables C2 and C3). Titanocene dichloride solutions from 1.0 to 3.0 mM tested without exogenous metabolic activation were positive for the induction of DNA single strand breaks in cultured hamster V79 cells as detected by the alkaline elution assay (Swenberg, 1981). Titanocene dichloride at a concentration of 10^{-3} M was reported to be weakly positive for the induction of unscheduled DNA synthesis in human fibroblast WI-38 cells (Mitchell, 1976). However, no differential growth inhibition was observed in DNA repair-deficient versus repair-competent *Escherichia coli* cells treated with 250 μg/plate titanocene dichloride (Rosenkranz and Poirier, 1979). Titanocene dichloride, tested at concentrations of 33 to 3,333 μg/plate, induced gene mutations in the *S. typhimurium* base pair substitution strain TA100 in the absence of S9, but was negative in several frameshift strains, with and without S9 activation (Haworth *et al.*, 1983; Appendix C, Table C1). No induction of mitotic recombination in *Saccharomyces cerevisiae* D3 was observed after treatment with titanocene dichloride in the presence or the absence of S9 (Simmon, 1979). There is no mutagenicity information available for metabolites or structural analogs of titanocene dichloride.

Study Rationale

Titanocene dichloride was nominated by the National Institute for Occupational Safety and Health for carcinogenic evaluation because of a potential for human exposure and to explore its toxicity and carcinogenicity by the oral route. A previous rat study had indicated that the compound caused tumors at the site of local intramuscular injection.

MATERIALS AND METHODS

Procurement and Characterization

Titanocene dichloride was obtained in two lots. Lot no. PB013180 from Pfaltz and Bauer, Inc. (Waterbury, CT) was used for the 14-day and 13-week studies. Strem Chemicals (Newbury Port, MA) provided lot no. 13574-S, which was used for the 2-year studies. Identity, purity, and stability analyses were conducted at the analytical chemistry laboratory, Midwest Research Institute (MRI), Kansas City, MO (Appendix G). The study chemical, a dark red, microcrystalline solid, was identified as titanocene dichloride by infrared and nuclear magnetic resonance spectroscopy. Lot no. PB013180 was greater than 98% pure, as determined by titration and elemental analysis. The purity of lot no. 13574-S was determined to be greater than 99% by titration, elemental analysis, and Karl Fischer water analysis. Stability studies performed by titration indicated that titanocene dichloride was stable as a bulk chemical for at least 2 weeks at temperatures to 60° C when protected from light.

Based on the stability study results, the bulk chemical was stored at 0° ± 5° C at the testing laboratory throughout the study period. The stability of the bulk chemical was monitored by elemental analysis and by titration periodically during all phases of the studies. No change in the study material was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing appropriate amounts of titanocene dichloride and corn oil (Appendix G, Table G1). Stability studies showed no decrease in titanocene dichloride concentration after storage of the suspensions for 2 weeks in the dark at 5° C or 25° C, or under simulated animal dosing conditions (open to air and light for 3 hours). During the studies, the dose formulations were stored at 0° ± 5° C for no longer than 2 weeks.

The study laboratory conducted periodic analyses of the titanocene dichloride dose formulations using high performance liquid chromatography or ultraviolet spectrophotometry as described in Appendix G. During the 2-year studies, the dose formulations were analyzed at approximately 8-week intervals and were within ± 10% of the target concentrations 96% (27/28) of the time (Appendix G, Table G4). The corn oil vehicle was analyzed for peroxides at monthly intervals; the peroxide content of the vehicle was within acceptable limits. Results of periodic referee analyses of the dose formulations performed by MRI were in agreement with the results from the study laboratory (Appendix G, Table G5).

14-Day Studies

Male and female F344/N rats were obtained from Charles River Breeding Laboratories (Kingston, NY). Male rats were quarantined for 6 to 8 days before the studies began; female rats were quarantined for 5 to 13 days. The rats were 7 weeks old at the beginning of the study.

Groups of 5 rats of each sex were administered 0, 62, 125, 250, 500, or 1,000 mg/kg titanocene dichloride in corn oil by gavage 5 days per week for a total of 12 dose days. Animals were housed five per cage. Water and feed were available *ad libitum*.

Animals were weighed prior to study initiation, on days 7 and 14, and at the end of the study. Observations for signs of toxicity were made twice daily throughout the studies. Animals found moribund and those surviving to the end of the study were killed, and blood was collected for hematology and clinical chemistry analyses. A complete necropsy was performed on all animals, including those dying before the end of the study. Brain, heart, right kidney, liver, lung, and thymus from all animals were weighed, as well as the right testis from all males. Portions of the heart, liver, lung, and spleen (frozen in liquid nitrogen and stored at −60° C) were taken for evaluation of tissue residues for titanium. Histopathologic examinations were performed on selected tissues and animals. Further experimental details are presented in Table 1.

13-Week Studies

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to titanocene dichloride and to determine the doses to be used in the 2-year studies.

Male and female F344/N rats were obtained from Frederick Cancer Research Facility (Frederick, MD). Animals were observed for 5 to 20 days, distributed to weight classes, and assigned to groups according to tables of random numbers. The rats were 8 to 9 weeks old when the study began. Further experimental details are provided in Table 1.

Groups of 10 rats of each sex were administered 0, 8, 16, 31, 62, or 125 mg/kg titanocene dichloride in corn oil by gavage 5 days per week for 13 weeks. Additional groups of 5 rats per sex received 0, 31, or 125 mg/kg titanocene dichloride on the same schedule and were used for the determination of tissue residues of titanium. Rats were housed five per cage. Feed and water were available *ad libitum*. Animals were observed twice daily for morbidity and mortality. Moribund animals were killed and necropsied. Individual animal weights were recorded at study initiation, weekly throughout the dosing period, and at the end of the study.

After 13 weeks, all surviving animals were killed. A complete necropsy was performed on all animals except those used for the determination of titanium levels in tissues. A complete histopathologic examination was performed on all core study animals in the control and 125 mg/kg dose groups, and selected tissues were examined from animals in the lower dose groups. Tissues and groups examined are listed in Table 1. Prior to processing, organ weights were determined for brain, heart, right kidney, liver, lung, thymus, and right testis (males) of all core study animals. The heart, liver, lungs, and spleen were collected from the animals predesignated for the titianium tissue level studies and were frozen and stored at −70° C. The frozen tissue samples were sent to MRI for analysis of titanium residues by inductively coupled plasma-atomic emission spectroscopy after a wet digestion procedure.

2-Year Studies

Study Design

Groups of 70 rats of each sex were administered 0, 25, or 50 mg/kg titanocene dichloride in corn oil by gavage at a dose volume of 5 mL/kg for 5 days per week for 104 weeks. Ten rats per dose group were evaluated (necropsy, organ weights, histopathology, tissue residues of titanium, and hematology analyses) after 15 months of chemical administration. In addition to these 70 animals, another ten rats per dose group were used in a separate research project that was not part of the 2-year carcinogenesis studies described in this report. One low-dose female in the separate research project died early and was included in the pathology analyses for the carcinogenesis studies. Thus, 61 low-dose females were used for the pathology and statistical evaluations for the 2-year studies, while 60 animals of each sex in the remaining dose groups were evaluated.

Source and Specification of Animals

Rats used in the 2-year studies were obtained at 4 weeks of age from the Frederick Cancer Research Facility (Frederick, MD). Males were quarantined for 11 days and females were quarantined for 13 days. During this time, animals were checked daily. To assess the health status of the rats, 5 animals per sex were killed prior to study initiation and were examined for infectious and parasitic diseases. The rats were about 6 weeks of age at the beginning of the study. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Rats were housed five per cage. Feed and water were available *ad libitum*. Further details of animal maintenance are given in Table 1. Racks were rotated in the room every two weeks, and cages were rotated from top to bottom within each group every two weeks.

Clinical Examinations and Pathology

All animals were observed twice daily, and clinical findings were recorded monthly or as necessary. Moribund animals were killed. Individual body weights were recorded prior to study initiation, once per week for the first 13 weeks of the studies, and every 4 weeks thereafter. Mean body weights were calculated for each group.

After 15 months, ten rats per dose group were killed for evaluation of organ weights, hematology parameters, tissue residues of titanium, and gross and microscopic pathology. Further details of the interim evaluations are given in Table 1.

The 104-week treatment period was followed by a one-week observation period, after which surviving animals were killed. A necropsy was performed on all animals including those found dead. Necropsies of all dosed rats were conducted within 7 working days of the end of the observation period; control animals were killed and necropsied within 9 working days of the end of the observation period. During necropsy, all organs and tissues were examined for grossly visible lesions. Portions of the heart, liver, lung, and spleen were collected from 10 randomly selected animals per dose group, frozen in liquid nitrogen, and shipped to MRI for analysis of titanium residues. Remaining tissues were preserved in 10% neutral buffered formalin and routinely processed for microscopic examination (embedded in paraffin, sectioned at 4 to 6 μm, and stained with hematoxylin and eosin). A complete histopathologic evaluation inclusive of gross lesions was performed on all animals. Tissues examined microscopically are listed in Table 1.

Upon completion of the microscopic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated. All tissues with a diagnosis of neoplasia; the liver, stomach, lungs, and nose from all male and female rats; the pancreas from all males; and all tissues from a randomly selected 10% of the control and high-dose rats were reevaluated microscopically by a quality assessment pathologist. In addition, sections of the spleen, thymus, duodenum, jejunum, bone marrow, and lymph nodes were reviewed for all rats to confirm the reported incidence of pigmentation.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the aforementioned tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative examples of potential chemical-related nonneoplastic lesions and neoplasms, lesions for which there was a difference in diagnosis between the study pathologist and reviewing pathologist, and lesions of general interest were selected by the chair for review by the PWG. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two-sided.

Calculation of Incidence

Tables A1 and B1 in the appendixes to this report present the incidence of neoplastic lesions in male and female rats. Tables A5 and B5 summarize the incidence of nonneoplastic lesions in male and female rats. The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (*e.g.*, skin or mammary tumors) prior to histologic sampling, or when lesions had multiple potential sites of occurrence (*e.g.*, mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence

The majority of tumors in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When tumors are incidental, this comparison of the time-specific tumor prevalences also provides a comparison of the time-specific tumor incidences (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one-sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. (For further discussion of these statistical methods, see Haseman, 1984.)

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control database (Haseman *et al.*, 1984; 1985) are included in the NTP reports for tumors appearing to show compound-related effects.

Analysis of Continuous Variables

The nonparametric multiple comparison procedures of Dunn (1964) or Shirley (1977) were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of organ weight data, clinical chemistry, and hematology. Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends and to determine whether Dunn's or Shirley's test was more appropriate for pairwise comparisons.

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with FDA Good Laboratory Practice Regulations (21 CFR Part 58). In addition, as study records were submitted to the NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so that all had been resolved or were otherwise addressed during the preparation of this Technical Report.

Genetic Toxicity

The genetic toxicity of titanocene dichloride was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and to induce sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. The protocols for these studies and tabular presentations of their findings are given in Appendix C.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Titanocene Dichloride

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
EG&G Mason Research Institute, Worcester, MA	Same as 14-day studies	Same as 14-day studies
Strain and Species		
F344/N rats	F344/N rats	F344/N rats
Animal Source		
Charles River Breeding Laboratories, Inc., Kingston, NY	Frederick Cancer Research Center, Frederick, MD	Same as 13-week studies
Time Held Before Study		
6 or 8 days (male) 5 or 13 days (female)	20 days (male) 7 or 12 days (female)	11 days (male) 13 days (female)
Age When Placed on Study		
7 weeks	8-9 weeks	6 weeks
Date of First Dose		
29 July 1981 (male) 1 July 1981 (female)	12 January 1982 (male) 2 February 1982 (female)	14 February 1983 (male) 16 February 1983 (female)
Duration of Dosing		
5 days/week for 12 dose days; sacrificed on day 17	5 days/week for 13 weeks	5 days/week for 104 weeks
Date of Last Dose		
13 August 1981 (male) 16 July 1981 (female)	15 April 1982 (male) 5-6 May 1982 (female)	12 February 1985 (male) 20 February 1985 (female)
Necropsy Dates		
14 August 1981 (male) 17 July 1981 (female)	15 April 1982 (male) 5-6 May 1982 (female)	15-month interim evaluation: 15-16 May 1984 (male) 23-24 May 1984 (female) 2-year (following a 1-week observation period): 20 February-4 March 1985 (male) 27 February-6 March 1985 (female)
Average Age at Necropsy		
9-10 weeks	21-22 weeks	111-112 weeks

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Titanocene Dichloride
(continued)

14-Day Studies	13-Week Studies	2-Year Studies
Size of Study Groups		
5 males and 5 females	10 males and 10 females; an additional 5 per sex in control, mid- and high-dose groups for tissue residue studies	60 males and 60 females; an additional 10 per dose group for 15-month interim evaluations
Method of Animal Distribution		
Animals randomized into dosed and control groups by weight so that cage weights were approximately equal (± 2 g)	Same as 14-day studies	Animals of each sex randomized into cage groups and then cage randomized to dosed and control groups by a table of random numbers
Animals per Cage		
5	5	5
Method of Animal Identification		
Ear punch	Same as 14-day studies	Same as 14-day studies
Feed		
NIH-07 open formula meal diet; (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Maximum Storage Time for Feed		
120 days after milling	Same as 14-day studies	Same as 14-day studies
Feeders		
Stainless steel, gang style (Scientific Cages, Inc., Bryan, TX), changed weekly; filled as needed	Same as 14-day studies; filled twice weekly, changed weekly	Same as 14-day studies; filled twice weekly, changed weekly
Water		
Automatic watering system, (Edstrom Industries, Inc., Waterford, WI), *ad libitum*	Same as 14-day studies	Same as 14-day studies
Cages		
Polycarbonate (Lab Products, Inc., Rochelle Park, NJ), changed twice weekly	Same as 14-day studies	Same as 14-day studies

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Titanocene Dichloride
(continued)

14-Day Studies	13-Week Studies	2-Year Studies
Bedding		
Aspen bed (American Excelsior Co., Baltimore, MD), changed twice weekly	Same as 14-day studies	Aspen bed or Beta Chips; changed twice weekly
Cage Filters		
Non-woven fiber (Snow Filtration, Cincinnati, OH)	Same as 14-day studies, changed every 2 weeks	Same as 14-day studies, changed every 2 weeks
Racks		
Stainless steel (Lab Products, Inc., Rochelle Park, NJ), changed once every two weeks	Same as 14-day studies	Same as 14-day studies
Animal Room Environment		
Temperature: 21.1°-24.4° C Humidity: 32%-78% Light: fluorescent, 12 hours/day Room air changes: 12-15 changes/hour	Temperature: 19.4°-25.0° C Humidity: 11%-50% Light: fluorescent, 12 hours/day Room air changes: greater than 12 changes/hour	Temperature: 22.4° ± 1.4° C Humidity: 45.2% ± 6.8% Light: fluorescent, 12 hours/day Room air changes: 10-12 changes/hour
Doses		
0, 62, 125, 250, 500, and 1,000 mg/kg in corn oil administered by gavage	0, 8, 16, 31, 62, and 125 mg/kg in corn oil administered by gavage	0, 25, or 50 mg/kg in corn oil administered by gavage
Storage Conditions for Dosing Solutions		
0 ± 5° C	Same as 14-day studies	Same as 14-day studies
Maximum Storage Time for Dosing Solutions		
2 weeks	2 weeks	2 weeks
Type and Frequency of Observation		
Observed twice daily for morbidity and mortality. Clinical observations recorded once daily. Individual body weights recorded at study initiation, on days 7 and 14, and at study termination.	Observed twice daily. Clinical observations recorded as necessary. Individual body weights recorded at study initiation, weekly during dosing, and at study termination.	Observed twice daily. Clinical observations recorded monthly or as necessary. Individual body weights were recorded at study initiation, weekly for the first 13 weeks, and every 4 weeks thereafter.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Titanocene Dichloride
(continued)

14-Day Studies	13-Week Studies	2-Year Studies
Necropsy Complete necropsy performed on all animals. Organ weights obtained for brain, heart, right kidney, liver, lung, thymus, and right testis (males). **Histopathology** Histopathologic examinations were performed on animals dying early and on selected organs in animals at terminal sacrifice. Tissues examined microscopically included kidney, liver, stomach, and testes for males given 0 to 500 mg/kg, and kidney, liver, and stomach for females given 500 mg/kg. Control females had kidney and liver examined, and females given 250 mg/kg had kidneys examined. Histological examinations were not performed for females given 62 or 125 mg/kg or for any animals given 1,000 mg/kg. The colon was also examined for males and females given 500 mg/kg. **Clinical Pathology** *Hematology*: hematocrit (automated and manual), hemoglobin, erythrocyte, leukocyte count and differential. *Clinical chemistry*: blood urea nitrogen; serum creatinine, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, total protein, albumin, globulin, albumin/globulin ratio, total and direct bilirubin, cholesterol, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, ornithine carbamoyl-transferase, sorbitol dehydrogenase, and pH *Urinalysis*: specific gravity and urine pH **Supplemental Studies** Sections of heart, liver, lungs, and spleen from control, 250 (males), and 500 mg/kg groups were evaluated for titanium residues.	**Necropsy** Complete necropsy performed on all animals except those used for the determination of titanium residues. Organ weights were recorded for brain, heart, right kidney, liver, lung, thymus, and right testis (males). **Histopathology** Histopathologic examinations were performed on all animals dying early, controls, and high-dose animals. Tissues examined included gross lesions and tissue masses, blood smear, mandibular and mesenteric lymph nodes, salivary glands, heart, esophagus, stomach, brain, sternebrae (including marrow), thyroid gland, parathyroid glands, small intestine, cecum, colon and rectum, liver, testes, epididymis, prostate gland, seminal vesicles, ovaries and uterus, lungs and bronchi, nasal cavity and nasal turbinates, thymus, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, pituitary gland, mammary gland, skin, and preputial or clitoral glands. Tissues were examined in animals in the lower dose groups. These included the spleen and stomach of females given 8 to 62 mg/kg. **Supplemental Studies** Sections of heart, liver, lung, and spleen from 5 animals of each sex in control, 31, and 125 mg/kg groups were evaluated for titanium residues.	**Necropsy** Complete necropsy performed on all animals. Organ weights of brain, heart, right kidney, liver, lung, and spleen were recorded. **Histopathology** Complete histopathologic examinations were performed on all control and high-dose animals evaluated at 15 months and on all animals in the core study. Tissues examined included: gross lesions and tissue masses (and regional lymph nodes), blood smear (15-month only), esophagus, cecum, colon, rectum, duodenum, ileum, jejunum, liver, pancreas, salivary glands, stomach, heart, adrenal glands, parathyroid glands, pituitary gland, thyroid gland, epididymis, preputial/clitoral gland, prostate gland, seminal vesicles, testes, ovaries, uterus, bone marrow, mandibular and mesenteric lymph nodes, spleen, thymus, skin, mammary gland, bone (sternebrae), brain, lungs and bronchi, nose, trachea, kidney, urinary bladder. Tissues examined in low-dose animals at the 15-month interim evaluations were gross lesions and bone marrow, brain, kidney, liver, lung, mandibular and mesenteric lymph nodes, spleen, and stomach. **Clinical Pathology** *Hematology*: 10 rats per dose were killed at 15 months for evaluation of hematology parameters including hematocrit, hemoglobin, erythrocyte count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet, reticulocyte, leukocyte count and differential. **Supplemental Studies** Sections of heart, liver, lung, and spleen were collected from 10 rats per dose at 15 months and at 2 years. These tissues were evaluated for titanium residues.

RESULTS

14-Day Studies

All rats in the 1,000 mg/kg dose group died by the ninth day of dosing. In addition, four of the five male and two of the five female rats receiving 500 mg/kg titanocene dichloride died before the end of the studies (Table 2).

There were dose-related decreases in final mean body weight in rats given 125, 250, or 500 mg/kg titanocene dichloride. Rats given 125 mg/kg gained approximately 28% less weight than controls and rats given 250 mg/kg gained 40% to 66% less weight than controls. Rats given 500 mg/kg lost weight during the studies. Body weight gains for rats given 1,000 mg/kg were not evaluated due to high mortality in these groups.

Clinical findings in all rats in the 500 and 1,000 mg/kg dose groups included ruffled fur and hunched posture. Many of these animals also had diarrhea, ataxic gait, and a red nasal discharge. All male rats given 125, 250, 500, or 1,000 mg/kg and all females given 1,000 mg/kg were lethargic during the studies.

TABLE 2
Survival and Mean Body Weights of Rats in the 14-Day Gavage Studies of Titanocene Dichloride

Dose (mg/kg)	Survival[a]	Mean Body Weights[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	170 ± 4	220 ± 5	50 ± 2	
62	5/5	170 ± 7	222 ± 8	52 ± 2	101
125	5/5	169 ± 4	206 ± 5	36 ± 2*	93
250	5/5	170 ± 4	187 ± 6**	17 ± 4**	85
500	1/5[c]	170 ± 3	107 ± 4**	-69 ± 2**	48
1,000	0/5[d]	170 ± 4	–	–	–
Female					
0	5/5	94 ± 4	134 ± 5	40 ± 2	
62	5/5	93 ± 3	138 ± 3	45 ± 2	103
125	5/5	94 ± 3	123 ± 5	29 ± 3*	91
250	5/5	94 ± 2	117 ± 2*	24 ± 2**	87
500	3/5[e]	94 ± 2	84 ± 10**	-8 ± 8**	63
1,000	0/5[f]	93 ± 2	–	–	–

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Number surviving/number initially in group
[b] Mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Day of death: 9, 9, 9, 16
[d] Day of death: 5, 7, 8, 8, 9; no data reported due to 100% mortality in this group
[e] Day of death: 13, 16
[f] Day of death: 4, 7, 7, 8, 9; no data reported due to 100% mortality in this group

Treatment-related decreases in absolute organ weights occurred for heart, kidney, lung, and thymus in male rats given 125 or 250 mg/kg titanocene dichloride, for liver in male rats given 250 mg/kg, and for heart and thymus in female rats given 125, 250, or 500 mg/kg (Tables D1a and D1b). Female rats also showed a significant chemical-related decrease in relative thymus weights, but the ratios were significant only for the 250 mg/kg group. These changes in organ weights were related to the decreased final body weights in treated groups.

There were mild chemical-related decreases in albumin, globulin, calcium, and total protein concentrations in male rats given 62, 125, or 250 mg/kg titanocene dichloride. Alanine aminotransferase (ALT) levels were significantly greater than the control for males given 62, 125, or 250 mg/kg (Table F2).

Significant decreases were present in total protein levels of females given 125, 250, or 500 mg/kg and in globulin levels of female rats given 250 or 500 mg/kg. There were significant chemical-related increases in ALT levels of females given 125, 250, or 500 mg/kg, albumin/globulin ratios and direct bilirubin levels of females given 250 or 500 mg/kg, and total bilirubin levels of females given 500 mg/kg. Hematocrit, hemoglobin and erythrocyte count values were decreased in female rats in the 500 mg/kg group (Table F1).

Tissue samples from males given 0, 250, or 500 mg/kg and from females given 0 or 500 mg/kg were analyzed for titanium residues. The highest levels of titanium were found in the spleen and liver (Table E1).

Lesions observed in treated animals included multifocal hepatocellular necrosis in two of the five males given 500 mg/kg titanocene dichloride, hepatocellular hypertrophy in all males and females given 500 mg/kg, and nephrosis (acute cortical tubule necrosis) in all rats given 500 mg/kg and in two of the five females given 250 mg/kg. There was hyperplasia of the forestomach epithelium of most male rats given 62, 125, 250, or 500 mg/kg and in three of the five females from the 500 mg/kg group. The severity of the hyperplasia in males increased with dose. Erosions and ulcers of the glandular stomach were also present in most treated males and in females from the 500 mg/kg dose group, and were associated with acute inflammation and regenerative hyperplasia.

13-Week Studies

One female rat given 125 mg/kg died during week 4 of treatment and one control male died during week 9 due to a cage maintenance accident (Table 3). The final mean body weights and the mean body weight changes for male and female rats in the 62 and 125 mg/kg dose groups were significantly lower than control (Table 3). Rats given 62 mg/kg weighed 8% to 10% less than controls at the end of the study, while rats given 125 mg/kg weighed 13% to 20% less than controls. Body weights for the other dose groups were similar to controls. All females given 125 mg/kg appeared thin and pale during the studies, and dyspnea was present in 2 of the 15 high-dose females.

Significant negative trends were observed for the absolute mean weights of the heart, liver, and thymus in male rats and of the thymus in female rats (Tables D2a and D2b). Mean heart weights were significantly lower than the control for all groups of dosed males except those given 31 mg/kg titanocene dichloride. Mean liver weight was significantly lower than control for males given 125 mg/kg only, and mean thymus weights were significantly lower than control for male and female rats in the 62 and 125 mg/kg dose groups. Males given 125 mg/kg had significantly lower relative liver and thymus weights, and males given 8 mg/kg had significantly lower relative heart weights than control. The absolute and relative organ weight changes were considered related to the lower body weights of treated animals.

TABLE 3
Survival and Mean Body Weights of Rats in the 13-Week Gavage Studies of Titanocene Dichloride

Dose (mg/kg)	Survival[a]	Mean Body Weights[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	14/15[c]	169 ± 3	372 ± 5	204 ± 5	
8	10/10	169 ± 4	371 ± 6	203 ± 5	100
16	10/10	169 ± 4	371 ± 8	202 ± 5	100
31	15/15	169 ± 2	365 ± 6	197 ± 6	98
62	10/10	170 ± 3	336 ± 9**	166 ± 6**	90
125	15/15	168 ± 2	296 ± 5**	128 ± 5**	80
Female					
0	15/15	131 ± 2	216 ± 2	85 ± 3	
8	10/10	131 ± 3	215 ± 2	84 ± 3	99
16	10/10	131 ± 2	211 ± 3	80 ± 4	97
31	15/15	131 ± 2	213 ± 3	82 ± 2	98
62	10/10	131 ± 2	199 ± 2**	68 ± 2**	92
125	14/15[d]	130 ± 2	189 ± 2**	59 ± 2**	87

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test
[a] Number surviving/number initially in group
[b] Mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Week of death: 9 (accidentally killed)
[d] Week of death: 4

Tissue samples of heart, liver, lung, and spleen from five male and five female rats given 0, 31, or 125 mg/kg were analyzed for titanium residues. The highest levels of titanium were found in the spleen and liver (Table E2).

Lesions associated with the administration of titanocene dichloride were observed only in the stomach of rats (Table 4). Hyperplasia and/or hyperkeratosis of the squamous epithelium of the forestomach involved the limiting ridge and the immediately adjacent area. It consisted of an increased number of basophilic cells in the basal cell layers and slight thickening of the overlying keratin layer. There were also superficial erosions of the glandular stomach mucosa resulting from focal necrosis of the surface epithelium and the subjacent gastric pits and upper portions of the gastric glands. An acute inflammatory response was usually associated with these lesions. Regenerative hyperplasia of the mucosa was also observed and was characterized by the replacement of mucous cells in the surface epithelium and gastric pits and parietal and chief cells in the gastric glands by less differentiated cells with enlarged nuclei. The affected glands had dilated lumens and irregular profiles. In a few scattered gastric glands of some rats, the parietal and chief cells were replaced by cells resembling pancreatic acinar cells (metaplasia). They had large nuclei with basophilic cytoplasm in the basal region of the cell and bright eosinophilic granules in the apical region.

Dose Selection Rationale
The doses for the 2-year studies, 25 mg/kg and 50 mg/kg, were chosen because of the reduced body weight changes and the increased incidences of stomach lesions in rats given 62 or 125 mg/kg during the 13-week studies.

TABLE 4
Lesions of the Stomach in Rats in the 13-Week Gavage Studies of Titanocene Dichloride

Organs and Diagnoses	Vehicle Control	8 mg/kg	16 mg/kg	31 mg/kg	62 mg/kg	125 mg/kg
Males						
Forestomach[a]	(10)	–[b]	–	–	–	(10)
Epithelial hyperplasia	0					3
Hyperkeratosis	0					4*
Glandular stomach	(10)	–	–	–	–	(10)
Hyperplasia[c]	0					7**
Erosion	0					9**
Fibrosis	0					4*
Metaplasia	0					5*
Inflammation	0					10**
Females						
Forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Epithelial hyperplasia	0	6**	6**	7**	6**	3
Hyperkeratosis	0	2	6**	6**	4*	8**
Glandular stomach	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia[c]	0	2	1	5*	6**	8**
Erosion	0	2	3	4*	4*	9**
Fibrosis	0	0	0	0	0	6**
Metaplasia	0	0	0	2	3	7**
Inflammation	0	5*	7**	6**	8**	9**

* Significantly different ($P \leq 0.05$) from the control group by Fisher exact test
** $P \leq 0.01$
[a] The number in parentheses is the number of animals with organ examined microscopically. More than one lesion may occur within the same organ.
[b] Tissue not examined for this dose group
[c] Lesion diagnosed as dysplasia by the laboratory pathologist.

2-Year Studies

15-Month Interim Evaluations

A dose-related decrease in mean necropsy body weight was observed in treated rats of both sexes (Tables D3a and D3b). This decrease was statistically significant for males given 25 or 50 mg/kg and for females given 50 mg/kg. Absolute and relative liver weights were significantly decreased in males given 50 mg/kg and significantly increased in females given 50 mg/kg. No other notable organ weight differences were observed.

Significant dose-related decreases in hematocrit, hemoglobin, mean cell volume, and mean cell hemoglobin values were observed for male and female rats given titanocene dichloride (Table F4). These changes in hematology values are consistent with a mild microcytic anemia of the type commonly accompanying various chronic inflammatory processes.

There were no apparent treatment-related neoplasms in male or female rats. Nonneoplastic lesions associated with the administration of titanocene dichloride occurred primarily in the stomach (Table 5) and were similar to those seen at two years. Accumulations of macrophages laden with blue-gray to gray-green pigment were seen in several organs of dosed male and female rats, but occurred predominantly in mesenteric lymph nodes and lungs. In a few selected animals, special stains were employed in an effort to further characterize the pigment. In these animals, the pigment was negative for the periodic acid-Schiff reaction and Perl's iron stain and was not believed to be hemosiderin or lipofuscin. The pigment was thought to contain titanium.

Body Weights

Mean body weights of male and female rats given 25 mg/kg titanocene dichloride were marginally lower (1% to 7%) than the control after week 14 (Tables 6 and 7 and Figure 1). Mean body weights of high-dose male rats were within 10% of controls for the first 33 weeks of dosing; after week 33, the mean body weights of high-dose males were 9% to 14% lower than those of the control animals. High-dose female rats had body weights within 10% of controls for the first 49 weeks of treatment; during the second year of the study, high-dose females had body weights 11% to 15% lower than controls. The final mean body weight for rats given titanocene dichloride were 96% and 91% for low- and high-dose males and 96% and 89% for low- and high-dose females.

Clinical Findings

The administration of titanocene dichloride was associated with a high incidence of abnormal respiratory sounds in male and female rats which were first noticed approximately eight months into the study. These low intensity respiratory sounds were audible only in close proximity to affected animals (auscultation was not performed). The respiratory sounds were probably due to exudate in the upper respiratory tract and were related to the pulmonary and nasal cavity lesions present in dosed animals. These sounds were noted in low-dose males (42/80), low-dose females (14/80), high-dose males (58/80), and high-dose females (24/80). In comparison, abnormal respiratory sounds were observed in only 1 of the 80 control males and none of the 80 control females.

Survival

Estimates of the probabilities of survival for male and female rats are shown in Table 8 and in the Kaplan-Meier curves in Figure 2. Low- and high-dose females had similar survival rates; although these rates were lower than the control value, the difference was not statistically significant. Treated males had survival rates significantly less than that of control males.

TABLE 5
Lesions of the Stomach in Rats at the 15-Month Evaluations in the 2-Year Gavage Studies of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Male			
Stomach, forestomach[a]	(10)	(10)	(10)
Acanthosis (Hyperplasia)	2 (1.5)[b]	8* (1.8)	4 (1.75)
Hyperkeratosis	2 (2.0)	5 (1.6)	4 (1.75)
Stomach, glandular	(10)	(10)	(10)
Erosion	0	6** (1.2)	1 (1.0)
Fibrosis	0	0	3 (2.7)
Hemorrhage	0	4* (1.0)	0
Hyperplasia, glandular	0	10** (1.6)	9** (1.3)
Inflammation, acute	0	3 (1.3)	3 (1.3)
Metaplasia, epithelial	0	4* (1.0)	4* (1.25)
Female			
Stomach, forestomach	(10)	(10)	(10)
Acanthosis (Hyperplasia)	0	3 (1.7)	8** (1.25)
Hyperkeratosis	0	3 (1.3)	7** (1.6)
Stomach, glandular	(10)	(10)	(10)
Erosion	0	5* (1.6)	7** (1.0)
Fibrosis	0	1 (2.0)	7** (2.1)
Hemorrhage	0	5* (1.8)	2 (1.0)
Hyperplasia, glandular	0	9** (1.4)	9** (1.7)
Inflammation, acute	0	6** (1.7)	7** (1.7)
Metaplasia, epithelial	0	4* (1.3)	6** (1.3)

* Significantly different ($P \leq 0.05$) from the control group by Fisher exact test
** $P \leq 0.01$
[a] The number in parentheses is the number of animals with organ examined microscopically. More than one lesion may occur within the same organ.
[b] Mean severity grades of lesions: 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride

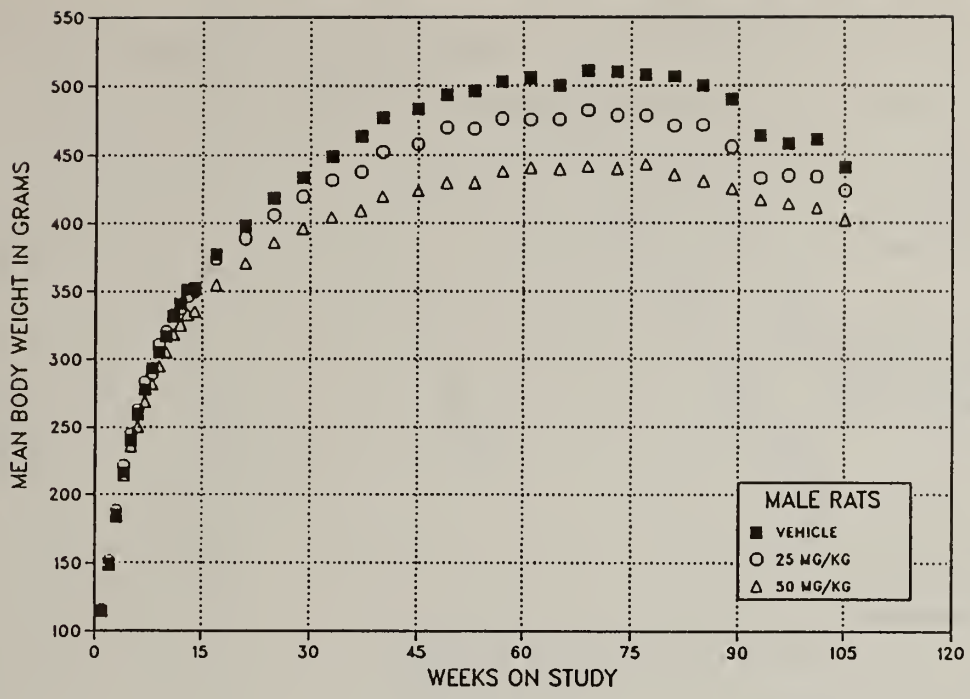
Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg		
	Av.Wt. (g)	No. of Survivors[a]	Av.Wt. (g)	Wt. (% of controls)	No. of Survivors[a]	Av.Wt. (g)	Wt. (% of controls)	No. of Survivors[a]
1	115	60	117	102	60	115	100	60
2	150	60	153	102	60	149	100	60
3	185	60	190	103	60	184	100	60
4	217	60	223	103	60	214	100	60
5	241	60	247	102	60	236	98	60
6	260	60	265	102	60	250	96	60
7	278	60	286	103	60	268	96	60
8	294	60	292	99	60	281	96	60
9	306	60	313	102	59	294	96	60
10	317	60	322	102	59	305	96	60
11	332	60	335	101	59	318	96	59
12	341	60	339	99	59	324	95	59
13	352	60	348	99	59	332	94	59
14	353	60	351	100	59	334	95	59
17	378	60	375	99	59	354	94	59
21	398	60	390	98	59	371	93	59
25	419	60	407	97	59	385	92	59
29	433	60	421	97	59	396	91	59
33	449	60	434	97	59	405	90	59
37	464	60	438	95	58	409	88	56
40	477	60	454	95	58	421	88	52
45	484	60	459	95	57	425	88	52
49	494	60	472	96	57	430	87	51
53	497	60	471	95	57	429	86	50
57	503	60	478	95	56	438	87	49
61	506	60	477	94	54	440	87	47
65	502	60	477	95	54	438	87	47
69	513	59	483	94	53	441	86	45
73	512	58	479	94	52	440	86	45
77	510	57	478	94	50	443	87	43
81	508	56	470	93	47	436	86	42
85	500	53	471	94	44	431	86	41
89	490	52	456	93	42	425	87	37
93	464	51	433	93	39	417	90	34
97	458	47	435	95	35	415	91	32
101	461	45	434	94	33	411	89	29
105	441	41	424	96	31	403	91	25
Terminal sacrifice		41			30			24
Mean for weeks								
1-13	261		264	101		252	97	
14-52	435		420	97		393	90	
53-105	490		462	94		429	88	

[a] Does not include interim evaluation animals

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride

Weeks on Study	Vehicle Control Av.Wt. (g)	Vehicle Control No. of Survivors[a]	25 mg/kg Av.Wt. (g)	25 mg/kg Wt. (% of controls)	25 mg/kg No. of Survivors[a]	50 mg/kg Av.Wt. (g)	50 mg/kg Wt. (% of controls)	50 mg/kg No. of Survivors[a]
1	101	60	102	101	61	103	102	60
2	121	60	123	101	61	125	104	60
3	139	60	139	100	61	142	103	60
4	151	60	152	101	61	153	102	60
5	161	60	159	99	61	162	101	60
6	172	60	171	99	61	172	100	60
7	180	60	178	99	61	179	100	60
8	187	60	186	100	61	185	99	60
9	194	60	191	99	61	191	98	60
10	200	60	198	99	61	197	98	60
11	204	60	203	99	61	200	98	60
12	206	60	203	99	61	200	97	60
13	209	60	208	100	61	204	98	60
14	210	60	210	100	61	205	98	60
17	221	60	219	99	61	212	96	60
21	228	60	223	98	61	214	94	60
25	234	60	232	99	61	219	93	60
29	240	60	235	98	60	226	94	60
33	244	60	239	98	60	228	93	60
37	251	60	245	98	60	232	93	57
41	262	60	257	98	60	243	93	57
45	267	59	259	97	60	241	90	56
49	275	59	269	98	60	249	90	56
53	285	58	273	96	59	253	89	53
57	292	57	280	96	59	256	88	53
61	302	56	284	94	59	257	85	52
65	310	56	295	95	58	265	85	52
69	317	56	303	96	56	272	86	49
73	329	56	310	94	55	283	86	45
77	330	56	316	96	54	289	88	44
81	332	55	319	96	53	293	88	44
85	339	55	326	96	51	301	89	43
89	343	52	332	97	47	305	89	42
93	341	48	326	96	42	302	89	40
97	345	46	323	94	38	298	86	37
101	345	41	329	95	34	306	89	32
105	337	37	324	96	30	299	89	32
Terminal sacrifice		37			30			31
Mean for weeks								
1-13	171		170	99		170	99	
14-52	243		238	98		227	93	
53-105	325		310	95		284	87	

[a] Does not include interim evaluation animals



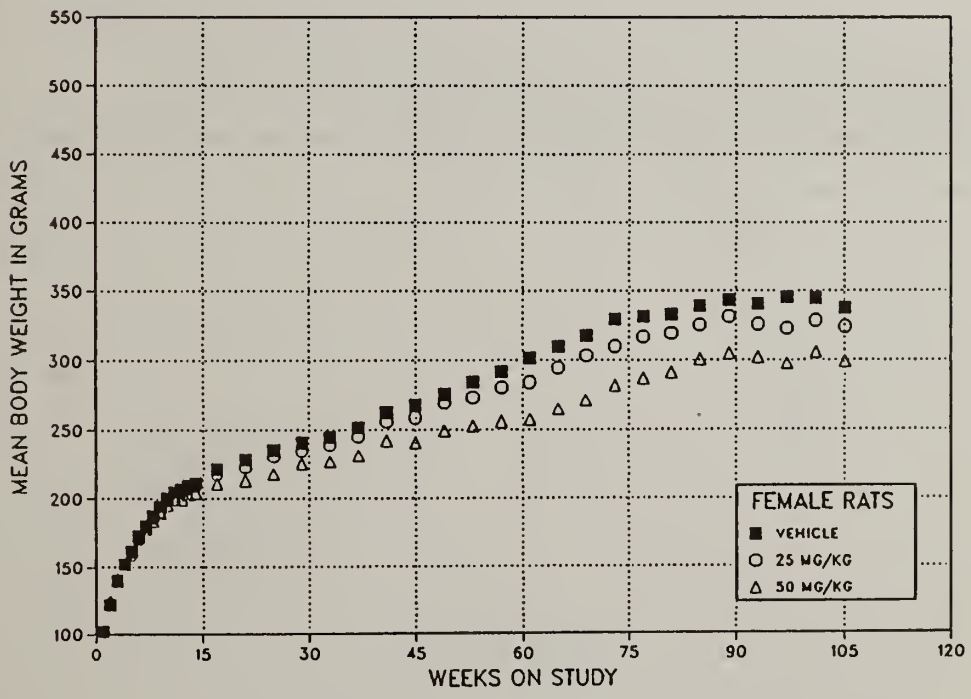


Figure 1
Growth Curves for Male and Female Rats Administered Titanocene Dichloride by Gavage for 2 Years

TABLE 8
Survival of Rats in the 2-Year Gavage Studies of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Male			
Animals initially in study	70	70	70
15-month interim evaluation[a]	10	10	10
Natural deaths	10	15	16
Moribund	8	14	17
Accidental deaths[a]	1	1	3
Animals surviving to study termination	41	30	24
Percent survival at end of study[b]	69	51	42
Mean survival days[c]	703	643	596
Survival P values[d]	0.002	0.046	0.002
Female			
Animals initially in study	70	71	70
15-month interim evaluation[a]	10	10	10
Natural deaths	3	5	7
Moribund	19	25	22
Accidental deaths[a]	1	1	0
Animals surviving to study termination	37[e]	30	31
Percent survival at end of study[b]	63	50	52
Mean survival days[c]	690	667	633
Survival P values[d]	0.164	0.229	0.194

[a] Censored from survival analyses.
[b] Kaplan-Meier determinations. Survival rates adjusted for accidental deaths and interim evaluations.
[c] Mean of all deaths (uncensored, censored, terminal sacrifice).
[d] The entry under the "control" column is the trend test (Tarone, 1975) result. Subsequent entries are the results of pairwise tests (Cox, 1972).
[e] One of these animals was found moribund on the last day of the study.

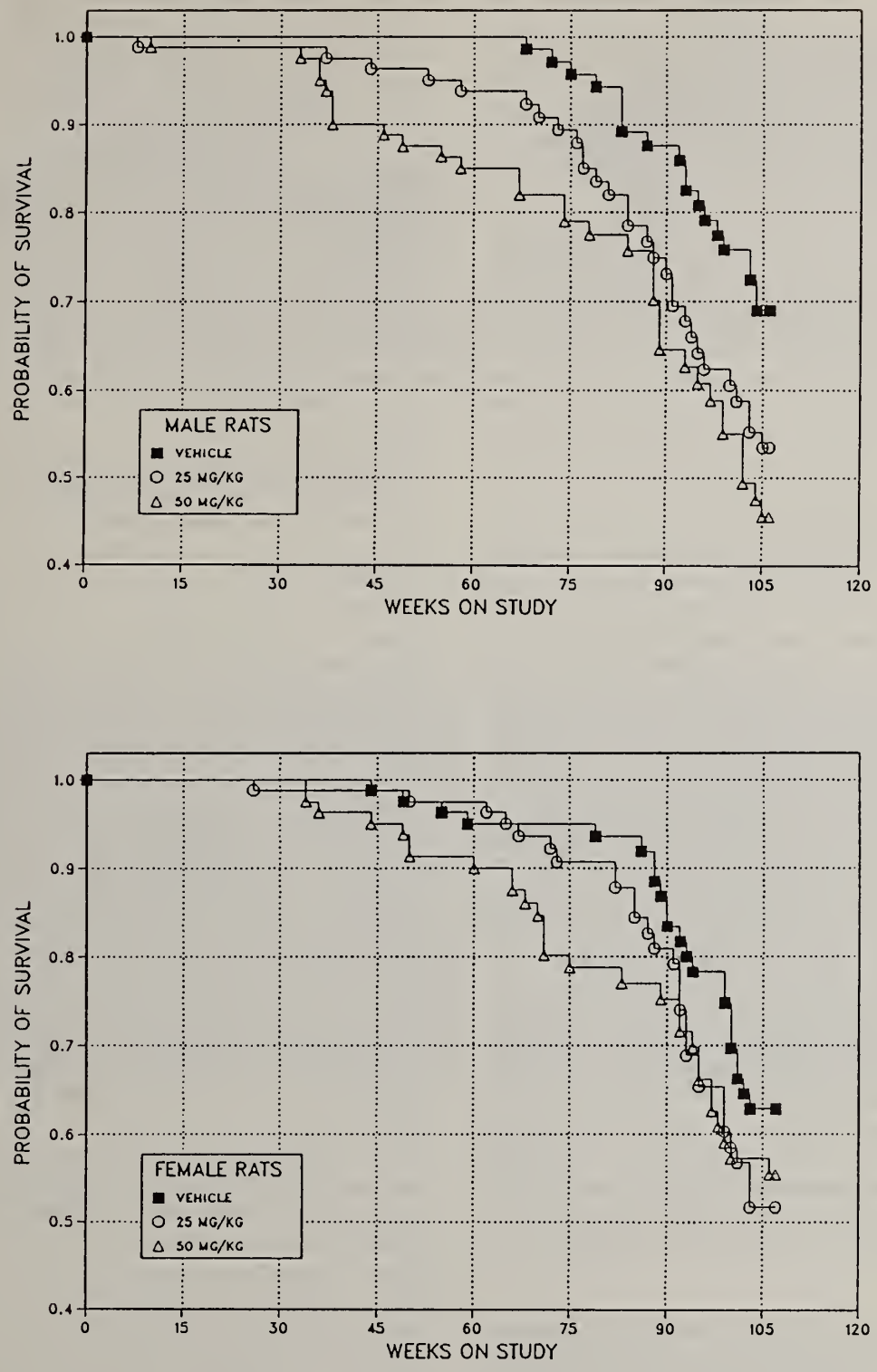


Figure 2
Kaplan-Meier Survival Curves for Male and Female Rats Administered Titanocene Dichloride by Gavage for 2 Years

Pathology and Statistical Analyses of Results of the 2-Year Studies

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the neoplasms mentioned in this section are presented in Appendixes A and B for male and female rats.

Stomach

The principal lesions associated with the administration of titanocene dichloride by oral gavage for up to 2 years occurred in the stomach (Table 9). The lesions were similar to those seen in the 13-week studies and at the 15-month interim evaluations, but were generally more extensive or severe.

Squamous cell papillomas occurred at low incidence in the forestomach of dosed rats, but not in controls (Table 9). In males, papillomas of the forestomach occurred in four low-dose and one high-dose animal, whereas in females papillomas were seen in one low-dose and two high-dose animals (Plate 1). A squamous cell carcinoma was seen in one low-dose male, which also had a squamous cell papilloma, and a basosquamous tumor was seen in one high-dose male. Squamous cell papilloma or carcinoma of the forestomach are uncommon neoplasms in control rats. The incidence of squamous cell papilloma or carcinoma combined in NTP historical controls is 9/2,735 for males and 8/2,748 for females with no more than two occurring in any single control group (Tables A4a and B4).

The papillomas were exophytic growths with squamous epithelium overlying a fibrovascular core, whereas the squamous cell carcinoma demonstrated invasion of the submucosa by cords and clusters of anaplastic squamous cells. The basosquamous tumor was a small circumscribed nodular mass projecting into the submucosa and consisting of basal cells and occasional small clusters of squamous cells. The tumor resembled benign basal cell neoplasms of the skin.

In the forestomach, hyperplasia of the epithelium (diagnosed as acanthosis in the 2-year studies) and hyperkeratosis usually occurred on or near the limiting ridge (junction of the forestomach and glandular stomach). There was variable thickening and folding of the stratified squamous epithelium due to an increase in cells composing the stratum spinosum, as well as an increase in basal cells, and accumulations of keratin on the surface (Plate 2). In some rats the squamous epithelium extended beyond the forestomach and covered parts of the adjacent glandular mucosa.

A spectrum of interrelated lesions occurred in the glandular stomach of dosed rats including erosions, inflammation, hyperplasia and metaplasia of the glandular epithelium (Plate 3), fibrosis of the lamina propria, fat proliferation, and pigmentation (Table 9). The glandular mucosa immediately adjacent to the limiting ridge of the forestomach was often the most severely affected. The erosions were superficial foci of necrosis with loss of the surface epithelium, gastric pits and fundic glands; the erosions generally did not extend across the muscularis mucosa into the submucosa. Infiltrates of neutrophils and mononuclear cells were present in the lamina propria and submucosa, and there was a variable increase in fibrous connective tissue in the lamina propria (Plate 4). Throughout the sections of stomach examined, there was atrophy of some fundic glands with concomitant hyperplasia or metaplasia of others. The atrophic glands exhibited loss of parietal and chief cells and were lined by nondescript cuboidal to columnar cells. In other glands the parietal and chief cells were replaced by less differentiated cuboidal cells which occasionally appeared multilayered. Two low-dose males and one low-dose female had a more severe form of hyperplasia (termed adenomatous hyperplasia) characterized by marked focal thickening of the mucosa and disorganization of the glands. The epithelium in these lesions was relatively uniform with minimal atypia and there was no extension through the muscularis mucosa. There was some uncertainty about the biological nature of these lesions, but it was the consensus of the Pathology Working Group that these lesions were not neoplasms. Single cells or clusters of cells similar to pancreatic acinar cells were present in scattered fundic glands (metaplasia). Mineralization was characterized by focal clusters of coarse basophilic granular material in areas of erosion and/or inflammation. The fat proliferation seen in dosed rats consisted of focal accumulations of well-differentiated adipocytes in the submucosa of the glandular stomach. This lesion also may represent a metaplasia or transdifferentiation of fibroblasts into adipocytes. The single lipoma found in a low-dose

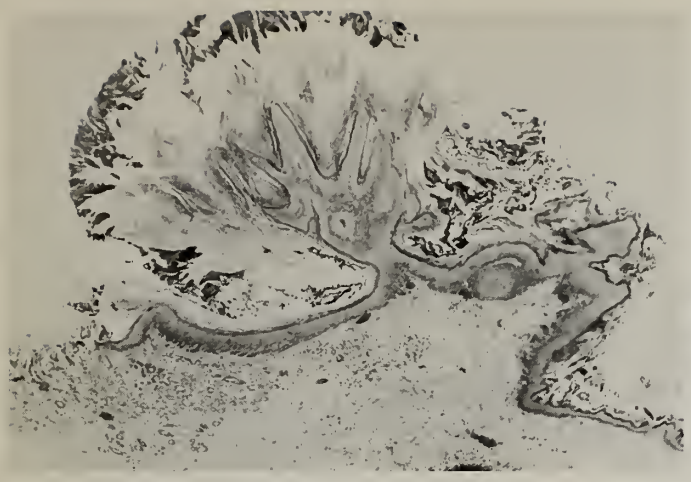


Plate 1
Squamous cell papilloma projects from the mucosal surface into the lumen of the forestomach of a high-dose female rat from the two-year titanocene dichloride studies. HE x 22.

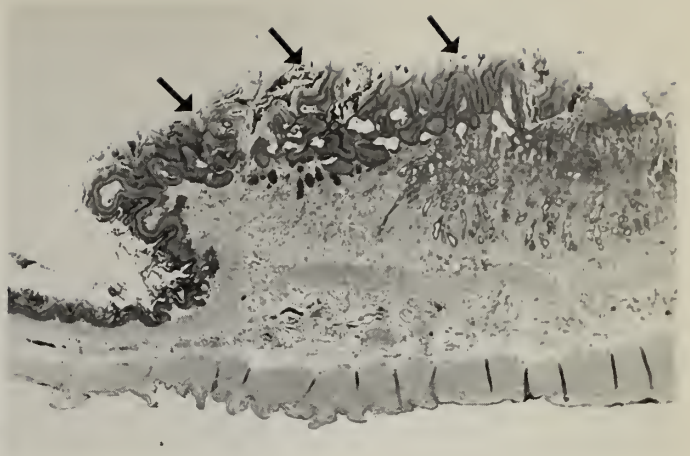


Plate 2
Acanthosis and hyperkeratosis in the mucosal epithelium (arrows) of the forestomach from a high-dose male rat from the two-year titanocene dichloride studies. HE x 22.

Plate 3
Glandular hyperplasia (G) and metaplasia (M) are present in the glandular stomach mucosa of a high-dose male rat from the two-year titanocene dichloride studies. HE x 164.

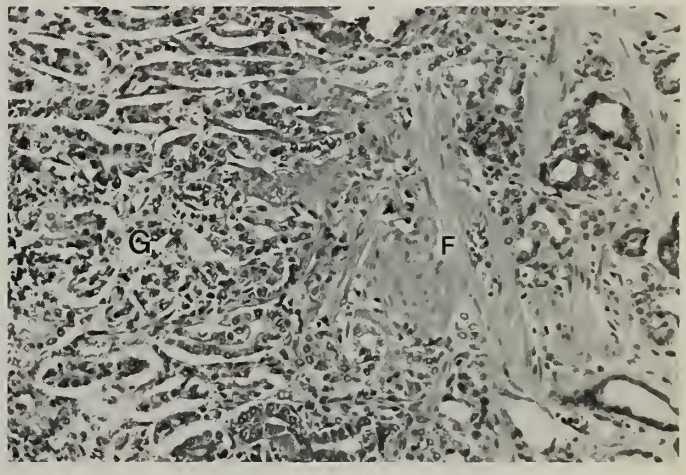


Plate 4
Fibrosis (F) in the glandular stomach lamina propria of a high-dose male rat from the two-year titanocene dichloride studies. Note adjacent glandular hyperplasia (G). HE x 164.

TABLE 9
Lesions of the Stomach of Rats in the 2-Year Gavage Studies of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Male			
Neoplasms[a]			
Stomach, forestomach			
Papilloma, squamous[b]			
Overall rates	0/60 (0%)	4/60 (7%)	1/60 (2%)
Adjusted rates[c]	0.0%	13.3%	4.2%
Terminal rates[d]	0/41 (0%)	4/30 (13%)	1/24 (4%)
First incidence (days)	–[e]	738 (T)	738 (T)
Logistic regression tests[f]	P=0.215	P=0.031	P=0.393
Squamous cell carcinoma[g]	0/60 (0%)	1/60 (2%)	0/60 (0%)
Basosquamous tumor	0/60 (0%)	0/60 (0%)	1/60 (2%)
Nonneoplastic Lesions[h]			
Stomach	(59)	(59)	(60)
Fat, proliferation	0	2 (1.0)[i]	14** (1.4)
Stomach, forestomach	(57)	(59)	(59)
Acanthosis (hyperplasia)	8 (1.9)	25** (1.7)	26** (1.8)
Hyperkeratosis	5 (2.0)	13* (2.1)	17** (2.3)
Stomach, glandular	(58)	(59)	(58)
Erosion	1 (2.0)	9** (1.4)	13** (1.4)
Fibrosis	0	30** (1.7)	37** (1.8)
Hyperplasia	0	10** (1.7)	24** (1.6)
Hyperplasia, adenomatous	0	2 (2.0)	0
Inflammation, acute	0	9** (1.8)	10** (1.5)
Metaplasia	0	26** (1.4)	36** (1.3)
Mineralization	0	2 (2.0)	1 (1.0)
Female			
Neoplasms[a]			
Stomach, forestomach			
Papilloma, squamous[j]			
Overall rates	0/60 (0%)	1/61 (2%)	2/60 (3%)
Adjusted rates	0.0%	3.3%	6.5%
Terminal rates	0/37 (0%)	1/30 (3%)	2/31 (6%)
First incidence (days)	–	743 (T)	743 (T)
Logistic regression tests	P=0.119	P=0.458	P=0.200

TABLE 9
Lesions of the Stomach of Rats in the 2-Year Gavage Studies of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Female (continued)			
Nonneoplastic Lesions[h]			
Stomach	(60)	(60)	(60)
Fat, proliferation	0	15** (1.0)	41** (2.5)
Stomach, forestomach	(60)	(60)	(60)
Acanthosis (hyperplasia)	11 (2.3)	20* (2.1)	27** (1.9)
Hyperkeratosis	10 (1.9)	23** (2.6)	21** (2.0)
Inflammation, acute	1 (1.8)	0	4 (1.8)
Stomach, glandular	(60)	(60)	(60)
Erosion	2 (2.0)	11* (1.4)	10* (1.2)
Fibrosis	0	39** (1.9)	51** (1.8)
Hyperplasia	0	24** (1.3)	23** (1.4)
Hyperplasia, adenomatous	0	1 (2.0)	0
Inflammation, acute	0	4 (1.5)	5* (1.4)
Metaplasia	0	36** (1.3)	51** (1.3)
Mineralization	0	7** (1.9)	2 (1.0)

* Significantly different ($P \leq 0.05$) from the control group by logistic regression analysis
** $P \leq 0.01$
(T)Terminal sacrifice
[a] Incidence rates for neoplasms are based on the number of rats necropsied.
[b] 2-year historical incidence for control groups receiving corn oil vehicle in NTP studies: 8/2,735 (0.3% ± 0.8%); range 0%-4%.
[c] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Not applicable; no tumors in animal group.
[f] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard these lesions as nonfatal.
[g] 2-year historical incidence for control groups receiving corn oil vehicle in NTP studies: 1/2,735 (0.04% ± 0.3%); range 0%-2%.
[h] Incidence rates for nonneoplastic lesions are based on the number of rats with stomach examined microscopically. The numbers in parentheses after an organ are the numbers of animals with organ examined microscopically.
[i] Mean severity grades of nonneoplastic lesions: 1=minimal, 2=mild, 3=moderate, 4=marked
[j] 2-year historical incidence for control groups receiving corn oil vehicle in NTP studies: 7/2,748 (0.3% ± 0.8%); range 0%-4%.

male was a large nodular mass of mature adipocytes. Whether it was related in any way to the fat proliferation is unknown. Macrophages containing blue-gray to gray-green pigment similar to that seen in other organs were also present in the lamina propria and submucosa of the glandular stomach.

Lung and Nose

There was a chemical-related increased incidence of acute inflammation of the nasal mucosa in male and female rats (Table 10). In males, fungal hyphae were often associated with the inflammatory lesions, whereas only a few females were similarly affected. Acute and/or granulomatous inflammation of the lung also occurred with a dose-related increase in treated rats. The inflammatory lesions were located near the terminal bronchioles and, occasionally, globules of yellow refractile material were present in the lesions. The foreign material may represent the corn oil vehicle. The lung and nose lesions were considered to be causally related to the reflux and aspiration of the gavage solution due to degenerative and inflammatory lesions in the stomach.

Infiltration of macrophages (histiocytes) in the lung also occurred more frequently in dosed rats than in controls. The macrophages were often located around small vessels and in alveoli near the pleural surface. In dosed rats, some macrophages contained blue-gray to gray-green pigment believed to be titanium.

TABLE 10
Nonneoplastic Lesions of the Lung and Nose in Rats in the 2-Year Gavage Studies of Titanocene Dichloride

Organ	Vehicle Control	25 mg/kg	50 mg/kg
Male			
Lung[a]	(60)	(60)	(60)
Infiltration, cellular, histiocytic	9 (1.3)[b]	46** (1.7)	49** (1.9)
Inflammation, acute	1 (2.0)	13** (1.8)	10** (2.8)
Inflammation, granulomatous	0	10** (1.2)	20** (1.4)
Pigmentation	0	34** (1.4)	50** (1.6)
Nose	(59)	(60)	(56)
Inflammation, acute	8 (2.1)	18** (2.2)	22** (2.5)
Female			
Lung	(60)	(61)	(60)
Infiltration, cellular, histiocytic	22 (1.5)	48** (1.4)	46** (1.3)
Inflammation, acute	2 (2.0)	4 (1.5)	9* (2.0)
Inflammation, granulomatous	0	3 (1.0)	8** (1.4)
Pigmentation	1 (1.0)	23** (1.0)	35** (1.1)
Nose	(60)	(61)	(60)
Inflammation, acute	7 (1.1)	13 (1.6)	15* (2.0)

* Significantly different ($P \leq 0.05$) from the control group by logistic regression analysis
** $P \leq 0.01$
[a] The number in parentheses is the number of animals with organ examined microscopically. More than one lesion may occur within the same organ.
[b] Mean severity grades of lesions: 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 11
Selected Lesions of the Liver in Rats in the 2-Year Gavage Studies of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Male			
Number examined	(60)	(59)	(60)
Basophilic focus	46	36	40
Clear cell focus	16	14	14
Eosinophilic focus	4	7	14**
Inflammation, granulomatous	0	16** (1.4)	14** (1.6)
Mixed cell focus	1	1	8*
Pigmentation	1 (2.0)[a]	39** (1.3)	41** (1.3)
Female			
Number examined	(60)	(61)	(60)
Inflammation, granulomatous	6 (2.0)	24** (1.8)	33** (1.3)
Pigmentation	3 (1.7)	45** (1.3)	50** (1.3)

* Significantly different (P≤0.05) from the control group by logistic regression analysis
** P≤0.01
[a] Mean severity grades of lesions: 1=minimal, 2=mild, 3=moderate, 4=mild

Liver

Granulomatous inflammation occurred at increased incidence in rats receiving titanocene dichloride (Table 11). The lesions were often located adjacent to or around portal areas and consisted of lymphocytes and macrophages. Pigment, similar to that found in other organs, was observed in some of the macrophages in these lesions as well as in individual Kupffer cells scattered throughout the lobules.

The incidences of mixed cell focus and eosinophilic focus were also increased in male rats, although those of basophilic focus and clear cell focus were not increased (Table 11). The various types of foci are distinguished on the basis of stain affinity, and the mixed cell foci usually contain a mixed population of basophilic and clear cells or eosinophilic and clear cells. The staining affinity of these cells usually corresponds to increases in cytoplasmic rough endoplasmic reticulum (basophilic cells), smooth endoplasmic reticulum (eosinophilic cells), or glycogen (clear cells). Whether these modest increases in mixed and eosinophilic foci are related to the administration of titanocene dichloride is uncertain.

There was a marginally increased incidence of hepatocellular adenomas in high-dose male rats relative to controls (control, 4/60; low-dose, 3/59; high-dose, 7/60); an hepatocellular carcinoma occurred in one high-dose male. The incidence of hepatocellular adenoma or carcinoma combined in NTP historical control male rats is 82/2,758 (3%) with as many as 7/50 in one control group (Table A4b). Because the increase was slight and the incidence of these neoplasms in the high-dose group was within the range of historical controls, they were not considered related to the administration of titanocene dichloride.

Mesothelium of Peritoneal Cavity and Tunica Vaginalis

Mesothelioma occurred in 3/60 low-dose and 4/60 high-dose male rats, but not in controls. The incidences of mesothelioma in NTP historical controls is 106/2,762 (3.8%) with as many as 6/50 in one control group (Table A4c). The marginal increase in mesotheliomas in dosed males is due largely to the lower than expected incidence in controls. Therefore, the small number of meso-

theliomas in dosed males was not considered related to the administration of titanocene dichloride.

Pigmentation of Various Organs

Small amounts of pigment similar to that seen in the stomach, lung, and liver as described above were seen within macrophages in various lymph nodes, spleen, and intestinal tract of dosed rats (Table A5 and Table B5). Since macrophages in the spleen and lymph nodes commonly contain hemosiderin, it was not always easy to discern those believed to contain titanium from those which contained only hemosiderin.

Clitoral Gland

There was a decreased incidence of clitoral gland adenomas or carcinomas in treated female rats (13/56; 9/55; 2/56). The incidence of clitoral gland adenomas or carcinomas in the control group was higher than the NTP historical control incidence of 138/2,763 (5%); previously no more than 9/50 had been seen in a given control group. Therefore, the apparent decreased incidence of these tumors in treated females may be spurious and not related to chemical administration.

Pituitary Gland

There was a decreased incidence of pituitary gland adenomas in treated females (29/59; 23/59; 16/60). The incidence of pituitary gland adenomas in NTP historical control females is 1,045/2,710 (39%) with as many as 32/49 in a single control group. In addition, the combined incidence of malignant tumors at all sites was also decreased in treated females (30/60; 25/61; 18/60). The incidence of malignant tumors at all sites in NTP historical control female rats is 1,018/2,763 (37%) with as many as 28/50 in one control group. The decreased incidences of pituitary gland tumors and of malignant tumors at all sites in the high dose females were marginally significant (P=0.041 and P=0.044) and were not considered to be clearly related to chemical administration.

Tissue Analysis for Titanium Residues

Titanium levels in the heart, liver, and lung ranged from approximately 15 to 39 ppm for males and 15 to 42 ppm for females (Table E3 and E4). However, the titanium levels in the spleen were much higher (100 to 180 ppm for males; 110 to 230 ppm for females) at both 15 months and 2 years.

Genetic Toxicology

Titanocene dichloride was tested at concentrations of up to 3,333 μg/plate for the induction of gene mutations in *Salmonella typhimurium* strain TA100, TA1535, TA1537, or TA98 both in the presence and in the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Appendix C, Table C1) using the procedures described by Haworth *et al.* (1983). A positive response was observed only for the base-substitution strain TA100 tested in the absence of S9; all other strain/activation combinations were negative. In cytogenetic tests with Chinese hamster ovary cells, titanocene dichloride was negative for the induction of sister chromatid exchanges (SCEs) and chromosomal aberrations, with and without metabolic activation (Appendix C, Tables C2 and C3). An increase in SCE frequency was observed at three of the four concentrations tested without S9, but these increases were not of sufficient magnitude to be considered a positive response and the results of the trend test were not significant. Doses in the SCE test ranged from 10 to 333 μg/mL without S9 and 33 to 1,000 μg/mL with S9. In the chromosomal aberration test without S9, doses ranged from 35 to 349 μg/mL and a delayed harvest protocol was used to allow sufficient metaphase cells to accumulate for analysis. In the presence of S9, doses of 162 to 750 μg/mL were tested; a precipitate formed at the two highest concentrations (349 and 750 μg/mL).

DISCUSSION AND CONCLUSIONS

Titanocene dichloride is an organometallic compound used as a catalyst in polymerization reactions; it has been shown to inhibit the growth of neoplasms in several model systems (Köpf-Maier and Köpf, 1986a; 1987). Repeated injection of titanocene dichloride or metallic titanium powder into the thigh muscle of rats produced local fibrosarcomas (Furst and Haro, 1969a,b; Furst, 1971). The NTP conducted 14-day, 13-week, and 2-year studies in F344/N rats to explore the potential toxicity and carcinogenicity of titanocene dichloride by oral administration.

In the NTP 14-day studies, mortality occurred in both sexes of rats receiving the two highest doses (500 and 1,000 mg/kg). Animals that died were observed to have had diarrhea, ataxia, acute renal tubular necrosis, and a spectrum of degenerative lesions in the stomach. Although the precise cause of death was not determined, it is likely that the rats died from the renal lesions complicated by serum electrolyte changes or an acid-base imbalance, or both, resulting from the gastric lesions. In the 13-week studies there was no dose-related mortality, but decreased body weight gain was seen in the 62 and 125 mg/kg dose groups. Toxic lesions were observed in the forestomach of 125 mg/kg animals, and erosions, fibrosis, hyperplasia, and metaplasia of the glandular stomach were present in females given 31, 62, or 125 mg/kg titanocene dichloride and in males receiving 125 mg/kg. Although histologic examination of the stomach was not performed for males in the 8, 16, 31, or 62 mg/kg dose groups, the stomach lesions in females from the 31 mg/kg dose groups were minimal in severity. Thus 50 mg/kg, a dose intermediate between 62 mg/kg (where body weight decrements were seen) and 31 mg/kg, was selected as the high dose for the 2-year studies.

In the 2-year studies, survival of male rats in the low- and high-dose groups was lower than controls, whereas survival of the dosed and control females was similar (males: control, 41/60; low-dose, 30/60; high-dose, 24/60; females: 37/60; 30/61; 31/60). The reduced survival of the males in the 50 mg/kg dose group was attributed to the presence of gastric lesions. Although the survival of the high-dose males was reduced relative to controls, over 50% were alive at week 97. Survival of low-dose males and all female dose groups was over 50% at 2 years. Survival, therefore, was considered adequate for detection of a carcinogenic response.

The gastric lesions observed in the forestomach and glandular stomach of animals in the 2-year studies were similar to those found in animals from the 13-week studies, but were generally more severe. The hyperplasia and hyperkeratosis of the squamous epithelium of the forestomach were more extensive and severe at the end of two years. In the glandular stomach, erosions with inflammation and regenerative hyperplasia of the glandular mucosa were seen in the 14-day studies, whereas metaplasia was first seen in 13-week studies. Fibrosis was generally minimal in the 13-week studies, whereas at 2 years some rats had focally extensive fibrosis with nearly complete replacement of the mucosa with fibrous tissue. Atrophy and/or loss of the fundic glands was also more severe and extensive after 2 years, although the condition was variable from animal to animal.

The metaplastic change in the fundic glands of the stomach has not been seen in previous NTP studies. Although the precise identity of the metaplastic cells was not determined, they closely resembled pancreatic acinar cells. The focal accumulations of adipocytes in the mucosa of the glandular stomach may also represent a metaplastic change. Metaplasia of several cell types (transdifferentiation) has occurred spontaneously and following chemical administration, especially in organs of endodermal embryonic origin. Examples of chemically induced metaplasia include pancreatic acinar cells in the liver of rats treated with polychlorinated biphenyls (Rao *et al.*, 1986), hepatocytes in the pancreas of rats given 2,6-dichloro-*p*-phenylenediamine (McDonald and Boorman, 1989), and adipocytes in the bladder wall of rats given C.I. Disperse Blue 1 (NTP, 1986b). Neoplasms have not been shown to develop from these types of metaplastic changes.

The forestomach and glandular stomach lesions found during these studies appear to be related to

contact with the parent compound, titanocene dichloride. Titanium, determined by plasma-atomic emission spectroscopy, was found to be present in other tissues including the heart, liver, lung, and spleen. This procedure measured total titanium and could not distinguish between atomic titanium and the parent compound or metabolites. Bioaccumulation occurred slowly with the maximum titanium concentrations in the organs tested being reached by 15 months. No further increase was observed at 2 years, indicating that steady state concentrations had been achieved. The spleen accumulated the highest levels of titanium. This suggests that the parent compound, titanocene dichloride, was toxic at the site of exposure, whereas the titanium-containing metabolite reaching other organs was relatively nontoxic. Recent studies by Köpf-Maier and Martin (1989) have shown that following the intraperitoneal injection of titanium dichloride, titanium accumulates in both the cytoplasm and nucleus of cells. Other studies suggest that the cytotoxicity of this compound results from the interaction with nucleic acid constituents (Toney *et al.*, 1986).

After two years of dosing, squamous cell papillomas of the forestomach were present in four low-dose and one high-dose male and in one low-dose and two high-dose female rats. Several of these papillomas occurred at the limiting ridge of the forestomach, however none were found in control rats. A squamous cell carcinoma occurred in a low-dose male, and a basosquamous tumor was seen in a high-dose male. Forestomach squamous cell papillomas have occurred in NTP historical vehicle control F344/N rats at a rate of 0.3% (8/2735 for males and 7/2748 for females) and forestomach squamous cell carcinomas occur at a rate of 0.04% (1/2735 for males and 1/2748 for females) (Tables A4a and B4). No more than two squamous cell carcinomas or papillomas occurred in any control group.

In 17 out of over 350 NCI/NTP 2-year rat studies, there was evidence of chemical-related tumor formation in the stomach. Sixteen chemicals showed evidence for tumor formation in the forestomach, but only two chemicals showed any evidence of tumor formation in the glandular stomach (Table 12). In 12 of these studies the chemical was administered by oral gavage (NCI, 1978a,e,f,g,h; NTP, 1985; 1986a,c,d; 1987a,b; 1990) and in the remaining five studies the chemical was administered in feed (NCI, 1978b,c,d,i; NTP, 1982). The mechanism for the formation of forestomach tumors is currently under investigation by scientists at NTP (Ghanayem *et al.*, 1986) and elsewhere (Kroes and Wester, 1986; Clayson *et al.*, 1990; Shibata *et al.*, 1990). Cytotoxicity and increased cell proliferation may enhance the expression of endogenous or exogenous cancer-causing events whether they result from mutations, DNA damage caused by chemicals, or alteration in the control of cell growth (Columbano *et al.*, 1981; Cohen and Ellwein, 1990).

Focal hyperplasia and papilloma of the stratified squamous epithelium are generally considered part of a sequential morphologic continuum often observed in initiation-promotion skin paint studies and with some forestomach carcinogens (Brown and Hardisty, 1990). Papillomas are distinguished from hyperplasia on the basis of a more complex structure and consist of branching papillary fronds with a core of fibrovascular connective tissue. Moreover, squamous cell carcinomas are occasionally seen arising from papillomas, although they may also arise from foci of squamous or basal cell hyperplasia or from foci of dysplasia (Brown and Hardisty, 1990). However, the probabilities and rates of progression from hyperplasia to papilloma, from papilloma to carcinoma, or from hyperplasia to carcinoma are usually unknown, and are probably influenced by many factors besides carcinogenicity. Although hyperplasia may occur as a primary response to a carcinogen, it may also occur as a regenerative response secondary to necrosis or increased cell loss from the epithelium. Moreover, persistent hyperplasia and cell proliferation associated with continued necrosis/cell loss may provide a "favorable environment" for the development of tumors, as has been demonstrated for the skin (Argyris, 1980; Argyris and Slaga, 1981) and bladder (Clayson and Pringle, 1966). DiPaola and Casto (1979) have used an *in vitro* assay to measure morphologic transformation of Syrian hamster embryo cells. Using this assay system to compare the relative transforming ability of a variety of metals, they found that direct exposure to nickel, cadmium, chromium, beryllium, or arsenic caused transformation, while exposure to iron, titanium, tungstate, zinc, aluminum, or amorphous nickel sulfide did not result in transformation. These experiments suggest that titanium may be less likely than other metals to cause transformation *in vivo*.

In NTP genetic toxicity studies, titanocene dichloride was positive for the induction of gene mutations in

TABLE 12
Chemicals Associated with the Induction of Stomach Neoplasms in Rats

Chemical Name	Technical Report Number	Route of Administration	Forestomach		Glandular Stomach	
			Male	Female	Male	Female
3-Chloro-2-methylpropene	300	gavage	X	X		
3-Chloromethylpyridine hydrochloride	095	gavage	X	X		
4-Chloro-o-phenylenediamine	063	feed	X	X		
C.I. Disperse Yellow 3	222	feed	X			
Clonitralid	091	feed				X
Cupferron	100	feed	X	X		
1,2-Dibromo-3-chloropropane	028	gavage	X	X		
1,2-Dibromoethane (ethylene dibromide)	086	gavage	X	X		
1,2-Dichloroethane	055	gavage	X			
1,3-Dichloropropene (Telone II)	269	gavage	X	X		
Diglycidyl resorcinol ether (DGRE)	257	gavage	X	X		
Dimethyl hydrogen phosphite	287	gavage	X	X		
Dimethylvinyl chloride (DMVC)	316	gavage	X	X		
Ethyl acrylate	259	gavage	X	X		
Glycidol	374	gavage	X	X		X
Pivalolactone	140	gavage	X	X		
Sulfallate	115	feed	X			

one *Salmonella typhimurium* strain tested in the absence of metabolic activation, but was negative in this strain in the presence of metabolic activation and in three other *S. typhimurium* strains with and without metabolic activation. Titanocene dichloride did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells. Other studies on titanium compounds do not indicate toxicity to the stomach of rodents. Titanocene dichloride and metallic titanium powder have been shown to produce local sarcomas following repeated intramuscular injection, but no tumors were observed at other sites (Furst and Haro, 1969a,b; Furst, 1971). In a 2-year inhalation study in rats in which animals were exposed to titanium dioxide at 0, 10, 50, 250 mg/m^2 for 6 hours/day, 5 days/week lung tumors were seen in the highest exposure group, and the authors attributed this response in part to massive dust accumulation in the lungs. Tumors at other sites including the stomach were not reported (Lee *et al.*, 1985). In an NCI 2-year rodent study, titanium dioxide administered in the feed at 0, 2.5%, and 5% gave no evidence of carcinogenic activity in either rats or mice (NCI, 1979).

Because of similarities in structure with *cis*-platinum (*cis*-diamminedichloroplatinum), titanocene dichloride has been investigated for and has demonstrated antitumor activity in several experimental models where the compound is given by intraperitoneal injection (Köpf-Maier *et al.*, 1980a,b, 1981; Köpf-Maier, 1988; 1989). Although slight decreases in the incidences of clitoral gland tumors, pituitary gland tumors, and malignant tumors (any site) were seen in female rats in the NTP study, it seems unlikely that these decreased incidences are related to the antitumor activity exhibited in the experimental models. Limitation of the cytotoxic effects of titanocene dichloride to the stomach in these gavage studies suggests that the biologically active form of the compound may not have reached other tissues at concentrations sufficient to inhibit tumor growth. The apparent decrease in clitoral gland tumors in dosed female rats is possibly due to the unexpected high rate in the concurrent controls as compared to historical controls. The decrease in pituitary gland tumors and malignant tumors may have been related to the reduced body weight in high-dose female rats. The biological significance of these effects is unknown.

In these 2-year studies of titanocene dichloride, the forestomach tumors in dosed male and female rats given titanocene dichloride by oral gavage were considered equivocal evidence of carcinogenic activity because: 1) the numbers of tumors were small and only slightly above historical controls; 2) there was no dose-related increase in tumor incidence in male rats; 3) the tumors were mostly benign (the only squamous cell carcinoma occurred in a low-dose male); and, 4) the biological potential of the relationship of hyperplasia and the papillomas is not clear.

Conclusions

Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity*[*] of titanocene dichloride in male F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas, squamous cell carcinoma, and basosquamous tumor benign. There was *equivocal evidence of carcinogenic activity* of titanocene dichloride in female F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas.

Nonneoplastic lesions associated with the administration of titanocene dichloride for up to 2 years included erosions and inflammation of the gastric mucosa, hyperplasia and metaplasia of the fundic glands with fibrosis of the lamina propria in the glandular stomach, and acanthosis (hyperplasia) and hyperkeratosis of the forestomach epithelium.

[*] Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of peer review comments and the public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF TITANOCENE DICHLORIDE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Disposition Summary			
Animals initially in study	70	70	70
15-month interim evaluation	10	10	10
Early deaths			
Dead	10	15	16
Moribund	8	14	17
Accident	1	1	3
Survivors			
Terminal sacrifice	41	30	24
Animals examined microscopically	60	60	60
Alimentary System			
Intestine large, colon	(56)	(54)	(57)
Intestine small, ileum	(55)	(52)	(57)
Liver	(60)	(59)	(60)
Hepatocellular carcinoma			1 (2%)
Hepatocellular adenoma	4 (7%)	2 (3%)	6 (10%)
Hepatocellular adenoma, multiple		1 (2%)	1 (2%)
Mesentery	(6)	(4)	(5)
Pancreas	(59)	(56)	(59)
Acinus, adenoma		4 (7%)	
Pharynx			(1)
Palate, papilloma squamous			1 (100%)
Stomach, forestomach	(57)	(59)	(59)
Basosquamous tumor benign			1 (2%)
Papilloma squamous		4 (7%)	1 (2%)
Squamous cell carcinoma		1 (2%)	
Stomach, glandular	(58)	(59)	(58)
Lipoma		1 (2%)	
Cardiovascular System			
Heart	(60)	(60)	(60)
Endocrine System			
Adrenal gland	(60)	(60)	(60)
Adrenal gland, cortex	(60)	(60)	(60)
Adenoma		1 (2%)	
Adrenal gland, medulla	(60)	(60)	(59)
Pheochromocytoma malignant	3 (5%)	1 (2%)	1 (2%)
Pheochromocytoma benign	15 (25%)	13 (22%)	8 (14%)
Bilateral, pheochromocytoma benign	5 (8%)	8 (13%)	9 (15%)
Islets, pancreatic	(58)	(56)	(59)
Adenoma	5 (9%)	5 (9%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Endocrine System (continued)			
Parathyroid gland	(56)	(54)	(51)
Adenoma		1 (2%)	
Pituitary gland	(56)	(59)	(56)
Pars distalis, adenoma	23 (41%)	18 (31%)	13 (23%)
Pars distalis, adenoma, multiple		2 (3%)	1 (2%)
Thyroid gland	(60)	(55)	(58)
C-cell, adenoma	12 (20%)	9 (16%)	9 (16%)
C-cell, carcinoma	1 (2%)		1 (2%)
Follicular cell, adenoma	1 (2%)	1 (2%)	
Follicular cell, carcinoma	1 (2%)		1 (2%)
General Body System			
None			
Genital System			
Epididymis	(60)	(58)	(60)
Preputial gland	(56)	(59)	(56)
Adenoma	3 (5%)	1 (2%)	3 (5%)
Carcinoma	1 (2%)		1 (2%)
Bilateral, adenoma	1 (2%)		
Testes	(60)	(60)	(60)
Bilateral, interstitial cell, adenoma	40 (67%)	41 (68%)	44 (73%)
Interstitial cell, adenoma	11 (18%)	9 (15%)	2 (3%)
Hematopoietic System			
Bone marrow	(59)	(58)	(60)
Lymph node	(60)	(59)	(60)
Lymph node, mandibular	(58)	(56)	(56)
Lymph node, mesenteric	(59)	(54)	(58)
Spleen	(58)	(56)	(59)
Thymus	(55)	(50)	(52)
Integumentary System			
Mammary gland	(42)	(36)	(41)
Fibroadenoma	4 (10%)	5 (14%)	2 (5%)
Fibroadenoma, multiple		1 (3%)	
Skin	(58)	(60)	(59)
Carcinoma, metastatic, Zymbal's gland	1 (2%)		
Keratoacanthoma	2 (3%)	1 (2%)	
Papilloma squamous	1 (2%)		1 (2%)
Sebaceous gland, adenoma		1 (2%)	
Subcutaneous tissue, fibroma	5 (9%)	2 (3%)	3 (5%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	2 (3%)	
Subcutaneous tissue, fibrous histiocytoma	1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Musculoskeletal System			
Bone	(60)	(60)	(60)
Osteoma		1 (2%)	
Osteosarcoma	1 (2%)		
Nervous System			
Brain	(60)	(59)	(60)
Granular cell tumor benign	1 (2%)		
Neoplasm NOS		1 (2%)	
Respiratory System			
Lung	(60)	(60)	(60)
Alveolar/bronchiolar adenoma	3 (5%)	2 (3%)	5 (8%)
Alveolar/bronchiolar carcinoma	1 (2%)		
Carcinoma, metastatic, Zymbal's gland	1 (2%)		
Carcinoma adenosquamous	1 (2%)		
Osteosarcoma, metastatic		1 (2%)	
Osteosarcoma, metastatic, bone	1 (2%)		
Nose	(59)	(60)	(56)
Schwannoma benign		1 (2%)	
Special Senses System			
Ear	(1)	(3)	(1)
Fibrosarcoma			1 (100%)
Zymbal's gland	(3)	(2)	(1)
Carcinoma	3 (100%)	2 (100%)	1 (100%)
Urinary System			
Kidney	(60)	(60)	(60)
Renal tubule, adenoma	1 (2%)		
Urinary bladder	(60)	(55)	(56)
Systemic Lesions			
Multiple organs[a]	(60)	(60)	(60)
Leukemia monocytic	1 (2%)		
Leukemia mononuclear	15 (25%)	12 (20%)	11 (18%)
Mesothelioma malignant		3 (5%)	4 (7%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichioride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Tumor Summary			
Total animals with primary neoplasms[b]	60	56	48
Total primary neoplasms	168	156	134
Total animals with benign neoplasms	59	55	48
Total benign neoplasms	137	134	112
Total animals with malignant neoplasms	24	19	19
Total malignant neoplasms	31	21	22
Total animals with secondary neoplasms[c]	3	1	
Total secondary neoplasms	3	1	
Total animals with neoplasms uncertain-benign or malignant		1	
Total uncertain neoplasms		1	

[a] The number in parentheses is the number of animals with any tissue examined microscopically.
[b] Primary tumors: all tumors except metastatic tumors
[c] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control

Number of Days on Study	4 7 4	4 9 9	5 2 0	5 5 0	5 7 7	5 7 8	5 7 9	6 0 5	6 3 9	6 4 7	6 4 7	6 5 9	6 7 0	6 8 1	6 9 0	7 1 6	7 1 7	7 2 2	7 2 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9
Carcass ID Number	1 2 5	1 6 5	1 1 5	0 1 5	0 4 4	0 3 5	0 8 4	0 7 4	1 5 5	0 5 4	1 4 5	0 3 3	1 2 4	1 4 4	1 4 3	1 1 3	0 5 3	0 1 3	1 5 4	0 2 4	0 3 1	0 8 3	0 9 3	1 3 3	0 2 3
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	+	+	+	+	A	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	A	+	A	A	A	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	M	+	+	A	+	+	+	+	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	A	+	+	+	+	A	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	A	+	+	+	+	A	+	A	A	A	A	+	A	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																								
Mesentery									+												+			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	M	+	+	+
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant											X					X								
Pheochromocytoma benign													X			X						X	X	
Bilateral, pheochromocytoma benign																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X								X				X							
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+
Pars distalis, adenoma							X			X	X		X	X	X					X		X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																				X		X		
C-cell, carcinoma																								
Follicular cell, adenoma										X														
Follicular cell, carcinoma																								

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 3 9	7 3 9	7 3 9	7 3 9	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 6	7 4 6	7 4 6
Carcass ID Number	0 6 4	1 0 4	1 1 2	1 3 2	0 1 1	0 1 2	0 5 2	0 6 1	0 6 2	0 6 3	1 0 2	1 1 1	1 1 2	1 5 3	0 2 1	0 2 2	0 4 1	0 4 2	0 4 3	0 7 2	0 7 3	1 0 1	0 8 1	0 8 2	0 9 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																				X					X
Mesentery					+	+																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																X									
Pheochromocytoma benign				X								X	X	X	X			X	X						X
Bilateral, pheochromocytoma benign					X																			X	
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma						X																			X
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X	X			X	X									X			X	X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X			X	X						X		X	X	X			X			X	
C-cell, carcinoma												X													
Follicular cell, adenoma																									
Follicular cell, carcinoma																							X		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 4 6	7 4 6	7 4 7	7 4 7	7 4 7	7 4 7	7 4 7	7 4 7	7 5 0	7 5 0	
Carcass ID Number	0 9 2	1 5 2	1 2 1	1 4 1	1 4 2	1 5 1	1 6 3	1 6 4	1 3 1	1 6 1	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	60
Intestine large	+	+	+	+	+	+	+	+	+	+	57
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	54
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	56
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	54
Intestine small	+	+	+	+	+	+	+	+	+	+	60
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	55
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatocellular adenoma					X		X				4
Mesentery				+	+						6
Pancreas	+	+	+	+	+	+	+	+	+	+	59
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach	+	+	+	+	+	+	+	+	+	+	59
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	57
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	58
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	60
Pheochromocytoma malignant											3
Pheochromocytoma benign	X			X			X				15
Bilateral, pheochromocytoma benign		X							X	X	5
Islets, pancreatic	+	+	+	+	+	+	+	+	M	+	58
Adenoma											5
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	56
Pituitary gland	+	+	+	+	+	+	+	+	+	+	56
Pars distalis, adenoma			X	X	X		X	X	X	X	23
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
C-cell, adenoma					X						12
C-cell, carcinoma											1
Follicular cell, adenoma											1
Follicular cell, carcinoma											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	4 7 4	4 9 9	5 2 0	5 5 0	5 7 7	5 7 8	5 7 9	6 0 5	6 3 9	6 4 7	6 4 7	6 5 9	6 7 0	6 8 1	6 9 0	7 1 6	7 1 7	7 2 2	7 2 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9
Carcass ID Number	1 2 5	1 6 5	1 1 5	0 1 5	0 4 4	0 3 5	0 8 4	0 7 4	1 5 5	0 5 4	1 4 5	0 3 3	1 2 4	1 4 4	1 4 3	1 0 3	0 5 3	0 1 3	1 5 4	0 2 4	0 3 1	0 8 3	0 9 3	1 3 3	0 2 3
General Body System																								
Tissue NOS																			+					
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Carcinoma																X								
Bilateral, adenoma																								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X		X	X				X			X	X	X			X	X	X		X	X	X	X	X
Interstitial cell, adenoma	X		X			X										X								
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	M	+	+	+	+	+	+	+	M	+	M	M	+	+	+	M	+	+	M	+	+	M	+	+	+
Fibroadenoma																					X				
Skin	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																								
Keratoacanthoma																								
Papilloma squamous																								
Subcutaneous tissue, fibroma		X																X						
Subcutaneous tissue, fibrosarcoma																								
Subcutaneous tissue, fibrous histiocytoma																			X					
Subcutaneous tissue, sarcoma																								
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma													X											
Skeletal muscle																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 3 9	7 3 9	7 3 9	7 3 9	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 6	7 4 6	7 4 6
Carcass ID Number	0 6 4	1 0 4	1 1 2	1 3 2	0 1 1	0 1 2	0 5 2	0 6 1	0 6 2	0 6 3	1 0 2	1 1 1	1 2 2	1 5 3	0 2 1	0 2 2	0 4 1	0 4 2	0 4 3	0 7 2	0 7 3	1 0 1	0 8 1	0 8 2	0 9 1
General Body System																									
Tissue NOS																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+
Adenoma								X													X				
Carcinoma																									
Bilateral, adenoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X		X	X	X	X	X			X	X	X	X	X		X	X	X		X	X	X	X	X	X
Interstitial cell, adenoma		X						X	X						X				X						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	M	M	+	+	+	+
Integumentary System																									
Mammary gland	M	+	M	+	+	+	+	+	+	+	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+
Fibroadenoma									X																X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, Zymbal's gland																		X							
Keratoacanthoma									X									X							
Papilloma squamous																								X	
Subcutaneous tissue, fibroma				X		X																			
Subcutaneous tissue, fibrosarcoma								X																	
Subcutaneous tissue, fibrous histiocytoma																									
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma																									
Skeletal muscle																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 4 6	7 4 6	7 4 7	7 4 7	7 4 7	7 4 7	7 4 7	7 4 7	7 5 0	7 5 0	
Carcass ID Number	0 9 2	1 5 2	1 2 1	1 4 1	1 4 2	1 5 1	1 6 3	1 6 4	1 3 1	1 6 1	Total Tissues/ Tumors
General Body System											
Tissue NOS											1
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	60
Preputial gland	+	+	+	M	+	+	+	M	+	+	56
Adenoma			X								3
Carcinoma											1
Bilateral, adenoma						X					1
Prostate	+	+	+	+	+	+	+	+	+	+	59
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	60
Testes	+	+	+	+	+	+	+	+	+	+	60
Bilateral, interstitial cell, adenoma	X	X	X			X			X		40
Interstitial cell, adenoma				X	X						11
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+		+	59
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	58
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	59
Spleen	+	+	+	+	+	+	+	+	+	+	58
Thymus	+	M	+	+	+	+	+	+	+	+	55
Integumentary System											
Mammary gland	+	M	+	M	M		M	M	+	+	42
Fibroadenoma									X		4
Skin	+	+	+	M	+	+	+	+	+	+	58
Carcinoma, metastatic, Zymbal's gland											1
Keratoacanthoma											2
Papilloma squamous											1
Subcutaneous tissue, fibroma		X									5
Subcutaneous tissue, fibrosarcoma											1
Subcutaneous tissue, fibrous histiocytoma											1
Subcutaneous tissue, sarcoma								X			1
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Osteosarcoma											1
Skeletal muscle								+			1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	4 7 4	4 9 9	5 2 0	5 5 0	5 7 7	5 7 8	5 7 9	6 0 5	6 3 9	6 4 7	6 4 7	6 5 9	6 7 0	6 8 1	6 9 0	7 1 6	7 1 7	7 2 2	7 2 7	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9
Carcass ID Number	1 2 5	1 6 5	1 1 5	0 1 5	0 4 4	0 3 5	0 8 4	0 7 4	1 5 5	0 5 4	1 4 5	0 3 3	1 2 4	1 4 4	1 4 3	1 1 3	0 5 3	0 1 3	1 5 4	0 2 4	0 3 1	0 8 3	0 9 3	1 3 3	0 2 3
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor benign																								
Spinal cord					+																			
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X	X									
Alveolar/bronchiolar carcinoma								X																
Carcinoma, metastatic, Zymbal's gland								X																
Carcinoma adenosquamous					X																			
Osteosarcoma, metastatic, bone													X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Ear						+																		
Eye												+											+	
Harderian gland												+											+	
Zymbal's gland								+																
Carcinoma								X																
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia monocytic																								
Leukemia mononuclear	X					X				X				X		X	X							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 3 9	7 3 9	7 3 9	7 3 9	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 5	7 4 6	7 4 6	7 4 6
Carcass ID Number	0 6 4	1 0 4	1 1 2	1 3 2	0 1 1	0 1 2	0 5 2	0 6 1	0 6 2	0 6 3	0 2 2	1 1 1	1 2 2	1 5 3	0 2 1	0 2 2	0 4 1	0 4 2	0 4 3	0 7 2	0 7 3	1 0 1	0 8 1	0 8 2	0 9 1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor benign																									X
Spinal cord																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									X
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, Zymbal's gland																									
Carcinoma adenosquamous																									
Osteosarcoma, metastatic, bone																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye											+			+											
Harderian gland											+			+											
Zymbal's gland																		+	+						
Carcinoma																		X	X						
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia monocytic																X									
Leukemia mononuclear		X						X							X	X				X	X	X			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 4 6	7 4 6	7 4 7	7 4 7	7 4 7	7 4 7	7 4 7	7 4 7	7 5 0	7 5 0	
Carcass ID Number	0 9 2	1 5 2	1 2 1	1 4 1	1 4 2	1 5 1	1 6 3	1 6 4	1 3 1	1 6 1	Total Tissues/ Tumors
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Granular cell tumor benign											1
Spinal cord											1
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma											3
Alveolar/bronchiolar carcinoma											1
Carcinoma, metastatic, Zymbal's gland											1
Carcinoma adenosquamous											1
Osteosarcoma, metastatic, bone											1
Nose	+	+	+	+	+	+	+	+	+	+	59
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Ear											1
Eye		+							+		6
Harderian gland		+							+		6
Zymbal's gland											3
Carcinoma											3
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Renal tubule, adenoma										X	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	60
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Leukemia monocytic											1
Leukemia mononuclear		X						X			15

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg

Number of Days on Study	0 5 4	2 5 3	3 0 6	3 7 0	4 0 0	4 0 6	4 7 4	4 8 4	5 0 9	5 2 6	5 3 6	5 3 7	5 5 3	5 6 7	5 8 2	5 8 3	6 0 3	6 1 1	6 2 8	6 3 4	6 3 5	6 5 0	6 5 3	6 5 9	6 6 7
Carcass ID Number	2 2 5	1 9 5	2 8 5	2 0 4	2 7 5	2 6 5	3 2 5	2 8 4	3 2 4	3 0 5	2 0 5	3 1 5	2 8 3	3 0 4	2 9 5	2 6 2	2 4 5	3 2 3	2 3 4	2 7 3	3 1 4	3 2 2	1 7 3	2 1 3	2 0 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine large, cecum	M	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+
Intestine large, colon	+	+	+	A	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+
Intestine large, rectum	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	A
Intestine small	A	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, duodenum	A	+	+	A	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, ileum	A	+	+	A	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	A
Intestine small, jejunum	A	+	+	A	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	A
Liver	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular adenoma, multiple																									
Mesentery																+									
Pancreas	A	+	+	A	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																		X							
Salivary glands	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Squamous cell carcinoma																									
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																					X				
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																	X								
Pheochromocytoma benign											X														
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	A	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	M	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	M
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X					X					X			X						X	
Pars distalis, adenoma, multiple																									
Thyroid gland	A	+	+	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma									X						X								X		X
Follicular cell, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	6 9 7	7 0 1	7 1 7	7 1 8	7 3 2	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9	7 3 9	7 3 9	7 3 9	7 4 0	7 4 0	7 4 0	7 4 0	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3
Carcass ID Number	2 5 4	2 4 4	2 3 3	2 7 2	3 1 3	2 2 4	2 7 1	2 8 2	2 9 3	2 9 4	1 7 1	1 7 2	1 8 3	2 5 3	2 9 2	2 2 2	2 3 1	2 4 1	2 4 3	1 8 1	1 9 2	1 9 3	2 0 3	2 1 2	2 2 1
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma												X											X		
Hepatocellular adenoma, multiple																									X
Mesentery										+											+				
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma												X								X					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous						X			X																
Squamous cell carcinoma						X																			
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X																					
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign		X				X	X		X			X				X		X	X			X		X	X
Bilateral, pheochromocytoma benign										X					X					X			X		
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X		X				X		X						
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X		X				X		X				X		X			X	X	X			X		
Pars distalis, adenoma, multiple											X														
Thyroid gland	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma			X						X						X								X		
Follicular cell, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	
Carcass ID Number	2 5 2	2 8 1	2 9 1	3 0 1	3 0 3	3 1 1	3 1 2	3 2 1	2 3 2	2 6 1	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	59
Intestine large	+	+	+	+	+	+	+	+	+	+	57
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	54
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	54
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	55
Intestine small	+	+	+	+	+	+	+	+	+	+	55
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	54
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	52
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	53
Liver	+	+	+	+	+	+	+	+	+	+	59
Hepatocellular adenoma											2
Hepatocellular adenoma, multiple											1
Mesentery							+				4
Pancreas	+	+	+	+	+	+	+	+	+	+	56
Acinus, adenoma										X	4
Salivary glands	+	+	+	+	+	+	+	+	+	+	59
Stomach	+	+	+	+	+	+	+	+	+	+	59
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	59
Papilloma squamous				X			X				4
Squamous cell carcinoma											1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	59
Lipoma											1
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	60
Adenoma											1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	60
Pheochromocytoma malignant											1
Pheochromocytoma benign						X					13
Bilateral, pheochromocytoma benign				X	X		X	X			8
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	56
Adenoma							X				5
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	54
Adenoma			X								1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	59
Pars distalis, adenoma							X		X	X	18
Pars distalis, adenoma, multiple	X										2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	55
C-cell, adenoma				X							9
Follicular cell, adenoma				X							1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	0 5 4	2 5 3	3 0 6	3 7 0	4 0 0	4 0 6	4 7 4	4 8 4	5 0 9	5 2 6	5 3 6	5 3 7	5 5 3	5 6 7	5 8 2	5 8 3	6 0 3	6 1 1	6 2 8	6 3 4	6 3 5	6 5 0	6 5 3	6 5 9	6 6 7
Carcass ID Number	2 2 5	1 9 5	2 8 5	2 0 4	2 7 5	2 6 5	3 2 5	2 8 4	3 2 4	3 0 5	2 0 5	3 1 5	2 8 3	3 0 4	2 9 5	2 6 2	2 4 5	2 2 3	3 3 4	2 7 3	3 1 4	3 2 2	1 7 3	2 1 3	2 0 1
General Body System																									
None																									
Genital System																									
Epididymis	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma												X													
Prostate	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma										X	X		X	X		X	X	X	X	X	X		X		X
Interstitial cell, adenoma							X	X				X			X							X		X	
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Lymph node, mesenteric	+	+	M	+	+	M	+	+	+	+	+	+	+	A	+	M	+	+	+	+	+	+	+	+	M
Spleen	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	M
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	M	+	+	M
Integumentary System																									
Mammary gland	M	+	+	M	M	+	+	M	M	+	+	+	M		+	M	+	+	+	+	M	+	M	+	M
Fibroadenoma																									
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebaceous gland, adenoma																					X				
Subcutaneous tissue, fibroma													X												
Subcutaneous tissue, fibrosarcoma			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplasm NOS																									
Spinal cord																							+		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	6 9 7	7 0 1	7 1 7	7 1 8	7 3 2	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 4 0	7 4 0	7 4 0	7 4 0	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3
Carcass ID Number	2 5 4	2 4 4	2 3 3	2 7 2	3 1 3	2 2 4	2 7 1	2 8 2	2 9 3	2 9 4	1 7 1	1 7 2	1 8 3	2 5 3	2 9 2	2 2 2	2 3 1	2 4 1	2 4 3	1 8 1	1 9 2	1 9 3	2 0 3	2 1 2	2 2 1
General Body System																									
None																									
Genital System																									
Epididymis	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Prostate	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X	X	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Interstitial cell, adenoma					X				X																
Hematopoietic System																									
Bone marrow	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	M	+	M	+	+	+	+	M
Integumentary System																									
Mammary gland	+	+	+	M	+	+	M	M	M	+	+	+	+	+	+	M	M	M	+	+	+	M	M	M	M
Fibroadenoma			X										X	X							X				
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteoma					X																				
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplasm NOS																				X					
Spinal cord																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	
Carcass ID Number	2 5 2	2 8 1	2 9 1	3 0 1	3 0 3	3 1 1	3 1 2	3 2 1	3 3 2	2 2 6	**Total Tissues/ Tumors**
General Body System											
None											
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	58
Preputial gland	+	+	+	+	+	+	+	+	+	+	59
Adenoma											1
Prostate	+	+	+	+	+	+	+	+	+	+	57
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	59
Testes	+	+	+	+	+	+	+	+	+	+	60
Bilateral, interstitial cell, adenoma	X	X	X		X	X		X	X	X	41
Interstitial cell, adenoma							X				9
Hematopoletic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	58
Lymph node	+	+	+	+	+	+	+	+	+	+	59
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	56
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	54
Spleen	+	+	+	+	+	+	+	+	+	+	56
Thymus	+	+	+	+	+	+	+	+	M	+	50
Integumentary System											
Mammary gland	+	+	+	M	+	M	+	+	+	+	36
Fibroadenoma									X		5
Fibroadenoma, multiple	X										1
Skin	+	+	+	+	+	+	+	+	+	+	60
Sebaceous gland, adenoma											1
Subcutaneous tissue, fibroma								X			2
Subcutaneous tissue, fibrosarcoma		X									2
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Osteoma											1
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	59
Neoplasm NOS											1
Spinal cord											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	0 5 4	2 5 3	3 0 6	3 7 0	4 0 0	4 0 6	4 7 4	4 8 4	5 0 9	5 2 6	5 3 6	5 3 7	5 5 3	5 6 7	5 8 2	5 8 3	6 0 3	6 1 1	6 2 8	6 3 4	6 3 5	6 5 0	6 5 3	6 9 3	6 6 7
Carcass ID Number	2 2 5	1 9 5	2 8 5	2 0 4	2 7 5	2 6 5	3 2 5	2 8 4	3 2 4	3 0 5	2 0 5	3 1 5	2 8 3	3 0 4	2 9 5	2 6 2	2 4 5	3 2 3	3 4 4	2 3 3	3 7 4	3 1 3	3 2 2	1 7 3	2 1 3	2 0 1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																										
Osteosarcoma, metastatic																								X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																			X							
Trachea	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear															+											
Eye							+	+		+																
Harderian gland							+																		+	
Zymbal's gland								+																		
Carcinoma								X																		
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear							X																	X		
Mesothelioma malignant																X										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	6 9 7	7 0 1	7 1 7	7 1 8	7 3 2	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 4 0	7 4 0	7 4 0	7 4 0	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3
Carcass ID Number	2 5 4	2 4 4	2 3 3	2 7 2	3 1 3	2 2 4	2 7 1	2 8 2	2 9 3	2 9 4	1 7 1	1 7 2	1 8 3	2 5 3	2 9 2	2 2 2	2 3 1	2 4 1	2 4 3	1 8 1	1 9 2	1 9 3	2 0 3	2 1 2	2 2 1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																				X					
Osteosarcoma, metastatic																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear									+																
Eye								+			+		+			+	+	+			+		+		
Harderian gland								+			+		+			+	+				+		+		
Zymbal's gland																									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X						X	X				X	X		X	X	X	X		
Mesothelioma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	
Carcass ID Number	2 5 2	2 8 1	2 9 1	3 0 1	3 0 3	3 1 1	3 1 2	3 2 1	2 3 2	2 6 1	**Total Tissues/ Tumors**
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma							X				2
Osteosarcoma, metastatic											1
Nose	+	+	+	+	+	+	+	+	+	+	60
Schwannoma benign											1
Trachea	+	+	+	+	+	+	+	+	+	+	58
Special Senses System											
Ear				+							3
Eye		+							+		13
Harderian gland									+		10
Zymbal's gland				+							2
Carcinoma				X							2
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Urinary bladder	+	+	+	+	+	+	+	+	+	+	55
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear				X							12
Mesothelioma malignant						X		X			3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg

Number of Days on Study	0 6 8	2 2 9	2 4 8	2 5 1	2 5 4	2 6 1	2 6 4	2 6 4	3 1 8	3 3 8	3 8 3	4 0 1	4 0 5	4 6 5	4 6 7	5 1 4	5 1 5	5 4 0	5 8 6	6 1 2	6 1 2	6 1 2	6 1 7	6 1 8	6 2 0
Carcass ID Number	4 7 5	4 6 4	4 5 5	4 6 5	3 4 5	4 0 5	3 7 5	4 5 4	3 7 4	3 6 5	3 3 5	3 8 5	3 3 5	4 3 4	4 8 5	4 1 3	3 9 4	3 9 3	4 0 4	3 9 2	4 5 3	4 6 2	3 8 2	4 1 5	3 4 2
Alimentary System																									
Esophagus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	A	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	M	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	A	+	+
Intestine small	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	M	+	+	+	+	+	A	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	+	A	A	+	A	+	+	+	+	A	+	+	+	+	+	A	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																				X					
Hepatocellular adenoma																									
Hepatocellular adenoma, multiple																									
Mesentery		+									+														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pharynx																									
Palate, papilloma squamous																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Basosquamous tumor benign																									
Papilloma squamous																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Pheochromocytoma malignant																									X
Pheochromocytoma benign																									
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	M	M	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M
Pituitary gland	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma														X		X	X					X			
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
C-cell, adenoma																					X	X			
C-cell, carcinoma																									
Follicular cell, carcinoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	6 3 9	6 4 7	6 6 2	6 7 5	6 9 0	6 9 0	7 0 8	7 0 9	7 1 1	7 2 2	7 3 4	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8
Carcass ID Number	3 3 3	4 7 4	4 4 4	3 9 1	3 7 2	3 8 1	4 4 3	4 1 4	4 1 2	4 0 3	3 4 1	3 3 1	3 5 1	3 5 3	3 5 5	3 6 1	3 6 2	3 7 1	4 0 1	4 0 2	4 1 1	4 2 1	4 2 3	4 2 5	4 3 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine large	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma							X	X			X		X	X	X		X								
Hepatocellular adenoma, multiple																									
Mesentery				+		+					+														
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Pharynx																									
Palate, papilloma squamous																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basosquamous tumor benign																		X							
Papilloma squamous																							X		
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign							X		X			X			X	X		X	X			X			
Bilateral, pheochromocytoma benign			X					X			X		X	X			X			X			X		X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X	X			X		X				X						X	X							X
Pars distalis, adenoma, multiple																									
Thyroid gland	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma		X	X		X					X	X										X				
C-cell, carcinoma					X																				
Follicular cell, carcinoma																		X							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	739	739	739	739	739	739	739	739	739	740	
Carcass ID Number	332	422	431	441	442	451	452	473	481	461	**Total Tissues/ Tumors**
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	58
Intestine large	+	+	+	+	+	+	+	+	+	+	57
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	55
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	57
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	54
Intestine small	+	+	+	+	+	+	+	+	+	+	58
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	53
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	57
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	51
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatocellular carcinoma											1
Hepatocellular adenoma											6
Hepatocellular adenoma, multiple							X				1
Mesentery											5
Pancreas	+	+	+	+	+	+	+	+	+	+	59
Pharynx								+			1
Palate, papilloma squamous								X			1
Salivary glands	+	+	+	+	+	+	+	+	M	+	59
Stomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	59
Basosquamous tumor benign											1
Papilloma squamous											1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	58
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	59
Pheochromocytoma malignant											1
Pheochromocytoma benign											8
Bilateral, pheochromocytoma benign											9
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	59
Adenoma			X								1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	51
Pituitary gland	+	+	+	+	+	+	+	+	+	+	56
Pars distalis, adenoma	X										13
Pars distalis, adenoma, multiple										X	1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	58
C-cell, adenoma									X		9
C-cell, carcinoma											1
Follicular cell, carcinoma											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	0 6 8	2 2 9	2 4 8	2 5 1	2 5 4	2 6 1	2 6 4	2 6 4	3 1 8	3 3 8	3 3 3	4 0 1	4 0 5	4 6 5	4 6 7	5 1 4	5 1 5	5 4 0	5 8 6	6 1 2	6 1 2	6 1 2	6 1 7	6 1 8	6 2 0
Carcass ID Number	4 7 5	4 6 4	4 5 5	4 6 5	3 4 5	4 0 5	3 7 5	4 5 4	3 7 4	3 6 5	3 3 5	3 8 5	4 3 5	4 3 4	4 8 5	4 1 3	3 9 4	3 9 3	4 0 4	3 9 2	4 5 3	4 6 2	3 8 2	4 1 5	3 4 2
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma													X		X		X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	M	M	M	+	M	M
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Keratoacanthoma																									
Papilloma squamous																									
Subcutaneous tissue, fibroma														X											
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	6 3 9	6 4 7	6 6 2	6 7 5	6 9 0	6 9 0	7 0 8	7 0 9	7 1 1	7 2 2	7 3 4	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8
Carcass ID Number	3 3 3	4 7 4	4 4 4	3 9 1	3 7 2	3 8 1	4 4 3	4 1 4	4 1 2	4 0 3	3 4 1	3 3 1	3 5 1	3 5 3	3 5 5	3 6 1	3 6 2	3 7 1	4 0 1	4 0 2	4 1 1	4 2 1	4 2 3	4 3 5	4 3 2
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X																						
Carcinoma					X																				
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interstitial cell, adenoma				X																					
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	+	M	M	M	+	M	M	M	+	+	+	+	+	M	+	+	+	+	M	M	M	M	+	M	+
Fibroadenoma																	X								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																		X							
Papilloma squamous																		X							
Subcutaneous tissue, fibroma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 3 9	7 4 0	
Carcass ID Number	3 3 2	4 2 2	4 3 1	4 4 1	4 4 2	4 5 1	4 5 2	4 7 3	4 8 1	4 6 1	Total Tissues/ Tumors
General Body System											
None											
Genital System											
Epididymis	+	+	+	+	+	+	+	+	+	+	60
Preputial gland	+	+	+	+	+	+	+	+	+	+	56
Adenoma				X			X				3
Carcinoma											1
Prostate	+	+	+	+	+	+	+	+	+	+	60
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	60
Testes	+	+	+	+	+	+	+	+	+	+	60
Bilateral, interstitial cell, adenoma		X	X	X	X	X	X	X	X	X	44
Interstitial cell, adenoma	X										2
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	60
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	56
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	58
Spleen	+	+	+	+	+	+	+	+	+	+	59
Thymus	+	+	+	+	+	+	+	+	+	+	52
Integumentary System											
Mammary gland	+	+	+	+	+	+	+	M	+	+	41
Fibroadenoma		X									2
Skin	+	+	+	+	+	+	+	+	+	+	59
Keratoacanthoma											1
Papilloma squamous											1
Subcutaneous tissue, fibroma		X									3
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	0 6 8	2 2 9	2 4 8	2 5 1	2 5 4	2 6 1	2 6 4	2 6 4	3 1 8	3 3 8	3 8 3	4 0 1	4 0 5	4 6 5	4 6 7	5 1 4	5 1 5	5 4 0	5 8 6	6 1 2	6 1 2	6 1 2	6 1 7	6 1 8	6 2 0
Carcass ID Number	4 7 5	4 6 4	4 5 5	4 6 5	3 4 5	4 0 5	3 7 5	4 5 4	3 7 4	3 6 5	3 3 5	3 8 5	3 3 5	4 3 4	4 3 5	4 8 3	4 1 3	3 9 4	3 9 3	4 0 4	3 9 2	4 5 3	4 6 2	3 8 2	4 1 5	3 4 2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																										
Nose	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear																										
Fibrosarcoma																										
Eye		+				+			+																	
Harderian gland									+																	
Zymbal's gland																										
Carcinoma																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear																					X					
Mesothelioma malignant																								X		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	6 3 9	6 4 7	6 6 2	6 7 5	6 9 0	6 9 0	7 0 8	7 0 9	7 1 1	7 2 2	7 3 4	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8	7 3 8
Carcass ID Number	3 3 3	4 7 4	4 4 4	3 9 1	3 7 2	3 8 1	4 4 3	4 1 4	4 1 2	4 0 3	3 4 1	3 3 1	3 5 1	3 5 3	3 5 5	3 6 1	3 6 2	3 7 1	4 0 1	4 0 2	4 1 1	4 2 1	4 2 3	4 2 5	4 3 2
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																X				X					X
Nose	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									+
Fibrosarcoma																									X
Eye						+										+		+	+			+			
Harderian gland						+										+		+	+			+			
Zymbal's gland																				+					
Carcinoma																				X					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	M	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X		X		X	X	X													X		
Mesothelioma malignant				X																				X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	739	739	739	739	739	739	739	739	739	740	
Carcass ID Number	32 2	42 2	43 1	44 1	44 2	45 1	55 2	47 3	48 1	46 1	Total Tissues/ Tumors
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma						X				X	5
Nose	+	+	+	+	+	+	+	+	+	+	56
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Ear											1
Fibrosarcoma											1
Eye							+				9
Harderian gland							+				7
Zymbal's gland											1
Carcinoma											1
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Urinary bladder	+	+	+	+	+	+	+	+	+	+	56
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear		X	X		X		X				11
Mesothelioma malignant											4

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Adrenal Gland (Medulla): Pheochromocytoma Benign			
Overall rates[a]	20/60 (33%)	21/60 (35%)	17/59 (29%)
Adjusted rates[b]	46.3%	65.1%	58.3%
Terminal rates[c]	18/41 (44%)	19/30 (63%)	12/24 (50%)
First incidence (days)	670	536	662
Life table tests[d]	P=0.078	P=0.089	P=0.123
Logistic regression tests[d]	P=0.152	P=0.126	P=0.198
Cochran-Armitage test[d]	P=0.335N		
Fisher exact test[d]		P=0.500	P=0.369N
Adrenal Gland (Medulla): Pheochromocytoma Malignant			
Overall rates	3/60 (5%)	1/60 (2%)	1/59 (2%)
Adjusted rates	6.5%	2.3%	2.8%
Terminal rates	1/41 (2%)	0/30 (0%)	0/24 (0%)
First incidence (days)	647	603	620
Life table tests	P=0.332N	P=0.407N	P=0.482N
Logistic regression tests	P=0.222N	P=0.305N	P=0.361N
Cochran-Armitage test	P=0.206N		
Fisher exact test		P=0.309N	P=0.316N
Adrenal Gland (Medulla): Pheochromocytoma (Benign or Malignant)			
Overall rates	22/60 (37%)	22/60 (37%)	18/59 (31%)
Adjusted rates	49.6%	65.9%	59.4%
Terminal rates	19/41 (46%)	19/30 (63%)	12/24 (50%)
First incidence (days)	647	536	620
Life table tests	P=0.103	P=0.122	P=0.149
Logistic regression tests	P=0.220	P=0.188	P=0.273
Cochran-Armitage test	P=0.272N		
Fisher exact test		P=0.575N	P=0.303N
Liver: Hepatocellular Adenoma			
Overall rates	4/60 (7%)	3/59 (5%)	7/60 (12%)
Adjusted rates	9.8%	10.0%	26.3%
Terminal rates	4/41 (10%)	3/30 (10%)	5/24 (21%)
First incidence (days)	738 (T)	738 (T)	708
Life table tests	P=0.042	P=0.643	P=0.057
Logistic regression tests	P=0.052	P=0.643	P=0.072
Cochran-Armitage test	P=0.198		
Fisher exact test		P=0.509N	P=0.264
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	4/60 (7%)	3/59 (5%)	8/60 (13%)
Adjusted rates	9.8%	10.0%	28.1%
Terminal rates	4/41 (10%)	3/30 (10%)	5/24 (21%)
First incidence (days)	738 (T)	738 (T)	612
Life table tests	P=0.022	P=0.643	P=0.032
Logistic regression tests	P=0.032	P=0.643	P=0.050
Cochran-Armitage test	P=0.124		
Fisher exact test		P=0.509N	P=0.181

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Lung: Alveolar/Bronchiolar Adenoma			
Overall rates	3/60 (5%)	2/60 (3%)	5/60 (8%)
Adjusted rates	6.6%	6.7%	20.8%
Terminal rates	1/41 (2%)	2/30 (7%)	5/24 (21%)
First incidence (days)	681	738 (T)	738 (T)
Life table tests	P=0.098	P=0.633N	P=0.132
Logistic regression tests	P=0.122	P=0.592N	P=0.169
Cochran-Armitage test	P=0.275		
Fisher exact test		P=0.500N	P=0.359
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall rates	5/60 (8%)	2/60 (3%)	5/60 (8%)
Adjusted rates	10.0%	6.7%	20.8%
Terminal rates	1/41 (2%)	2/30 (7%)	5/24 (21%)
First incidence (days)	577	738 (T)	738 (T)
Life table tests	P=0.306	P=0.343N	P=0.340
Logistic regression tests	P=0.404	P=0.226N	P=0.473
Cochran-Armitage test	P=0.573		
Fisher exact test		P=0.219N	P=0.628N
Mammary Gland: Fibroadenoma			
Overall rates	4/60 (7%)	6/60 (10%)	2/60 (3%)
Adjusted rates	9.8%	19.2%	8.3%
Terminal rates	4/41 (10%)	5/30 (17%)	2/24 (8%)
First incidence (days)	738 (T)	717	738 (T)
Life table tests	P=0.553	P=0.196	P=0.599N
Logistic regression tests	P=0.567N	P=0.200	P=0.599N
Cochran-Armitage test	P=0.292N		
Fisher exact test		P=0.372	P=0.340N
Pancreas: Adenoma			
Overall rates	0/59 (0%)	4/56 (7%)	0/59 (0%)
Adjusted rates	0.0%	12.1%	0.0%
Terminal rates	0/40 (0%)	3/30 (10%)	0/23 (0%)
First incidence (days)	–[e]	611	–
Life table tests	P=0.468	P=0.036	–
Logistic regression tests	P=0.525	P=0.043	–
Cochran-Armitage test	P=0.621		
Fisher exact test		P=0.053	–
Pancreatic Islets: Adenoma			
Overall rates	5/58 (9%)	5/56 (9%)	1/59 (2%)
Adjusted rates	10.8%	16.7%	4.3%
Terminal rates	2/39 (5%)	5/30 (17%)	1/23 (4%)
First incidence (days)	577	738 (T)	738 (T)
Life table tests	P=0.257N	P=0.459	P=0.246N
Logistic regression tests	P=0.186N	P=0.519	P=0.156N
Cochran-Armitage test	P=0.088N		
Fisher exact test		P=0.606	P=0.099N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	23/56 (41%)	20/59 (34%)	14/56 (25%)
Adjusted rates	50.1%	52.2%	39.7%
Terminal rates	17/39 (44%)	13/30 (43%)	6/24 (25%)
First incidence (days)	579	400	465
Life table tests	P=0.481N	P=0.402	P=0.493N
Logistic regression tests	P=0.159N	P=0.478N	P=0.171N
Cochran-Armitage test	P=0.044N		
Fisher exact test		P=0.274N	P=0.054N
Preputial Gland: Adenoma			
Overall rates	4/56 (7%)	1/59 (2%)	3/56 (5%)
Adjusted rates	10.8%	2.0%	11.1%
Terminal rates	4/37 (11%)	0/30 (0%)	2/24 (8%)
First incidence (days)	738 (T)	537	662
Life table tests	P=0.574	P=0.245N	P=0.585
Logistic regression tests	P=0.518N	P=0.191N	P=0.640
Cochran-Armitage test	P=0.411N		
Fisher exact test		P=0.166N	P=0.500N
Preputial Gland: Adenoma or Carcinoma			
Overall rates	5/56 (9%)	1/59 (2%)	4/56 (7%)
Adjusted rates	12.8%	2.0%	14.0%
Terminal rates	4/37 (11%)	0/30 (0%)	2/24 (8%)
First incidence (days)	716	537	662
Life table tests	P=0.516	P=0.163N	P=0.510
Logistic regression tests	P=0.541N	P=0.115N	P=0.597
Cochran-Armitage test	P=0.420N		
Fisher exact test		P=0.092N	P=0.500N
Skin (Subcutaneous Tissue): Fibroma			
Overall rates	5/60 (8%)	2/60 (3%)	3/60 (5%)
Adjusted rates	11.0%	5.3%	9.1%
Terminal rates	3/41 (7%)	1/30 (3%)	1/24 (4%)
First incidence (days)	499	553	465
Life table tests	P=0.494N	P=0.339N	P=0.607N
Logistic regression tests	P=0.298N	P=0.215N	P=0.392N
Cochran-Armitage test	P=0.275N		
Fisher exact test		P=0.219N	P=0.359N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	6/60 (10%)	4/60 (7%)	3/60 (5%)
Adjusted rates			
Terminal rates	4/41 (10%)	2/30 (7%)	1/24 (4%)
First incidence (days)	499	306	465
Life table tests	P=0.413N	P=0.532N	P=0.500N
Logistic regression tests	P=0.173N	P=0.304N	P=0.293N
Cochran-Armitage test	P=0.189N		
Fisher exact test		P=0.372N	P=0.245N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma			
Overall rates	7/60 (12%)	4/60 (7%)	3/60 (5%)
Adjusted rates	15.7%	10.2%	9.1%
Terminal rates	5/41 (12%)	2/30 (7%)	1/24 (4%)
First incidence (days)	499	306	465
Life table tests	P=0.312N	P=0.426N	P=0.403N
Logistic regression tests	P=0.113N	P=0.220N	P=0.215N
Cochran-Armitage test	P=0.116N		
Fisher exact test		P=0.264N	P=0.161N
Stomach (Forestomach): Squamous Papilloma			
Overall rates	0/60 (0%)	4/60 (7%)	1/60 (2%)
Adjusted rates	0.0%	13.3%	4.2%
Terminal rates	0/41 (0%)	4/30 (13%)	1/24 (4%)
First incidence (days)	–	738 (T)	738 (T)
Life table tests	P=0.215	P=0.031	P=0.393
Logistic regression tests	P=0.215	P=0.031	P=0.393
Cochran-Armitage test	P=0.391		
Fisher exact test		P=0.059	P=0.500
Testes: Adenoma			
Overall rates	51/60 (85%)	50/60 (83%)	46/60 (77%)
Adjusted rates	92.6%	96.1%	100.0%
Terminal rates	37/41 (90%)	28/30 (93%)	24/24 (100%)
First incidence (days)	474	474	405
Life table tests	P=0.004	P=0.031	P=0.004
Logistic regression tests	P=0.030	P=0.195	P=0.042
Cochran-Armitage test	P=0.144N		
Fisher exact test		P=0.500N	P=0.177N
Thyroid Gland (C-Cell): Adenoma			
Overall rates	12/60 (20%)	9/55 (16%)	9/58 (16%)
Adjusted rates	29.3%	23.7%	26.8%
Terminal rates	12/41 (29%)	4/30 (13%)	2/24 (8%)
First incidence (days)	738 (T)	509	612
Life table tests	P=0.383	P=0.594	P=0.405
Logistic regression tests	P=0.549	P=0.512N	P=0.538
Cochran-Armitage test	P=0.300N		
Fisher exact test		P=0.398N	P=0.347N
Thyroid Gland (C-Cell): Adenoma or Carcinoma			
Overall rates	13/60 (22%)	9/55 (16%)	9/58 (16%)
Adjusted rates	31.7%	23.7%	26.8%
Terminal rates	13/41 (32%)	4/30 (13%)	2/24 (8%)
First incidence (days)	738 (T)	509	612
Life table tests	P=0.460	P=0.516N	P=0.476
Logistic regression tests	P=0.467N	P=0.430N	P=0.583N
Cochran-Armitage test	P=0.225N		
Fisher exact test		P=0.315N	P=0.268N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Zymbal's Gland: Carcinoma			
Overall rates	3/60 (5%)	2/60 (3%)	1/60 (2%)
Adjusted rates	6.7%	5.2%	4.2%
Terminal rates	2/41 (5%)	1/30 (3%)	1/24 (4%)
First incidence (days)	605	484	738 (T)
Life table tests	P=0.379N	P=0.612N	P=0.482N
Logistic regression tests	P=0.258N	P=0.474N	P=0.406N
Cochran-Armitage test	P=0.223N		
Fisher exact test		P=0.500N	P=0.309N
All Organs: Leukemia (Monocytic or Mononuclear)			
Overall rates	15/60 (25%)	12/60 (20%)	11/60 (18%)
Adjusted rates	30.9%	36.3%	35.3%
Terminal rates	9/41 (22%)	10/30 (33%)	5/24 (21%)
First incidence (days)	474	474	612
Life table tests	P=0.356	P=0.514	P=0.407
Logistic regression tests	P=0.512N	P=0.481N	P=0.537N
Cochran-Armitage test	P=0.217N		
Fisher exact test		P=0.331N	P=0.253N
All Organs: Mesothelioma Malignant			
Overall rates	0/60 (0%)	3/60 (5%)	4/60 (7%)
Adjusted rates	0.0%	8.7%	13.6%
Terminal rates	0/41 (0%)	2/30 (7%)	2/24 (8%)
First incidence (days)	–	583	618
Life table tests	P=0.017	P=0.081	P=0.023
Logistic regression tests	P=0.027	P=0.105	P=0.035
Cochran-Armitage test	P=0.049		
Fisher exact test		P=0.122	P=0.059
All Organs: Benign Tumors			
Overall rates	59/60 (98%)	55/60 (92%)	48/60 (80%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	41/41 (100%)	30/30 (100%)	24/24 (100%)
First incidence (days)	474	400	405
Life table tests	P=0.021	P=0.052	P=0.025
Logistic regression tests	P=0.396	P=0.556	P=0.572
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.103N	P=0.001N
All Organs: Malignant Tumors			
Overall rates	24/60 (40%)	19/60 (32%)	19/60 (32%)
Adjusted rates	45.4%	50.1%	54.5%
Terminal rates	13/41 (32%)	13/30 (43%)	9/24 (38%)
First incidence (days)	474	306	612
Life table tests	P=0.239	P=0.513	P=0.258
Logistic regression tests	P=0.474N	P=0.270N	P=0.576N
Cochran-Armitage test	P=0.194N		
Fisher exact test		P=0.223N	P=0.223N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
All Organs: Benign or Malignant Tumors			
Overall rates	60/60 (100%)	56/60 (93%)	48/60 (80%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	41/41 (100%)	30/30 (100%)	24/24 (100%)
First incidence (days)	474	306	405
Life table tests	P=0.031	P=0.054	P=0.035
Logistic regression tests	P=0.348N	P=0.853N	P=0.470N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.059N	P<0.001N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group.

TABLE A4a
Historical Incidence of Neoplasms of the Forestomach in Male F344/N Rats Receiving Corn Oil Vehicle

Study	Incidence in Controls		
	Squamous Papilloma	Squamous Cell Carcinoma	Squamous Papilloma or Squamous Cell Carcinoma
Historical Incidence at EG&G Mason Research Institute[a]			
Bromoform	0/50	0/50	0/50
Hexachloroethane	0/50	0/50	0/50
Phenylbutazone	0/50	0/50	0/50
Probenecid	0/50	0/50	0/50
Diglycidyl resorcinol ether	0/50	0/50	0/50
Diglycidyl resorcinol ether	0/50	0/50	0/50
1,2-dichloropropane	1/50	0/50	1/50
Chlorodibromomethane	0/50	0/50	0/50
N-butyl chloride	1/50	0/50	1/50
Bromodichloromethane	0/49	1/49	1/49
Total	2/499 (0.4%)	1/499 (0.2%)	3/499 (0.6%)
Standard deviation	0.8%	0.6%	1.0%
Range	0%–2%	0%–2%	0%–2%
Overall Historical Incidence[a]			
Total	8/2,735[b] (0.3%)	1/2,735 (0.04%)	9/2,735[b] (0.3%)
Standard deviation	0.8%	0.3%	0.8%
Range	0%–4%	0%–2%	0%–4%

[a] Toxicology Data Management System compilation (data as of 22 December 1989), and Carcinogenesis Bioassay Data System compilation (data as of 6 March 1990)
[b] Includes one papilloma NOS.

TABLE A4b
Historical Incidence of Neoplasms of the Liver in Male F344/N Rats Receiving Corn Oil Vehicle

Study	Incidence in Controls		
	Hepatocellular Adenoma or Neoplastic Nodule	Hepatocellular Carcinoma	Hepatocellular Adenoma, Neoplastic Nodule, or Hepatocellular Carcinoma
Historical Incidence at EG&G Mason Research Institute[a]			
Bromoform	4/50	1/50	5/50
Hexachloroethane	1/50	1/50	2/50
Phenylbutazone	0/50	0/50	0/50
Probenecid	1/50	0/50	1/50
Diglycidyl resorcinol ether	1/50	0/50	1/50
Diglycidyl resorcinol ether	1/50	0/50	1/50
1,2-dichloropropane	1/50	2/50	3/50
Chlorodibromomethane	3/50	0/50	3/50
N-butyl chloride	2/50	1/50	3/50
Bromodichloromethane	1/50	0/50	1/50
Total	15/500[b] (3.0%)	5/500 (1.0%)	20/500[b] (4.0%)
Standard deviation	2.4%	1.4%	3.0%
Range	0%–8%	0%–4%	0%–10%
Overall Historical Incidence[a]			
Total	65/2,758[c] (2.4%)	18/2,758 (0.7%)	82/2,758[c] (3.0%)
Standard deviation	2.8%	1.1%	3.0%
Range	0%–14%	0%–4%	0%–14%

[a] Toxicology Data Management System compilation (data as of 22 December 1989; data for hepatocellular adenoma includes neoplastic nodules) and Carcinogenesis Bioassay Data System compilation (data as of 6 March 1990)
[b] CBDS data includes 9 neoplastic nodules
[c] CBDS data includes 52 neoplastic nodules and 2 hepatocellular adenomas

TABLE A4c
Historical Incidence of Mesothelioma in Male F344/N Rats Receiving Corn Oil Vehicle

Study	Incidence in Controls
Historical Incidence at EG&G Mason Research Institute[a]	
Bromoform	2/50
Hexachloroethane	1/50
Phenylbutazone	3/50
Probenecid	5/50
Diglycidyl resorcinol ether	0/50
Diglycidyl resorcinol ether	0/50
1,2-dichloropropane	3/50
Chlorodibromomethane	2/50
N-butyl chloride	2/50
Bromodichloromethane	2/50
Total	20/50 (40.0%)
Standard deviation	3.0%
Range	0%–10%
Overall Historical Incidence[a]	
Total	106/2,762 (3.8%)
Standard deviation	2.5%
Range	0%–12%

[a] Toxicology Data Management System compilation (data as of 22 December 1989) and Carcinogenesis Bioassay Data System compilation (data as of 6 March 1990) for mesothelioma benign, malignant, and NOS

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Disposition Summary			
Animals initially in study	70	70	70
15-month interim evaluation	10	10	10
Early deaths			
Dead	10	15	16
Moribund	8	14	17
Accident	1	1	3
Survivors			
Terminal sacrifice	41	30	24
Animals examined microscopically	60	60	60
Alimentary System			
Intestine large, cecum	(54)	(54)	(55)
Pigmentation			1 (2%)
Intestine large, colon	(56)	(54)	(57)
Parasite	4 (7%)	2 (4%)	4 (7%)
Pigmentation			1 (2%)
Intestine large, rectum	(54)	(55)	(54)
Parasite	5 (9%)		1 (2%)
Intestine small, duodenum	(59)	(54)	(53)
Pigmentation		23 (43%)	36 (68%)
Intestine small, ileum	(55)	(52)	(57)
Pigmentation			3 (5%)
Lymphoid tissue, hyperplasia			1 (2%)
Intestine small, jejunum	(53)	(53)	(51)
Pigmentation		1 (2%)	14 (27%)
Lymphoid tissue, hyperplasia			1 (2%)
Liver	(60)	(59)	(60)
Angiectasis			1 (2%)
Basophilic focus	46 (77%)	36 (61%)	40 (67%)
Clear cell focus	16 (27%)	14 (24%)	14 (23%)
Congestion			1 (2%)
Cyst	1 (2%)		
Degeneration, cystic	1 (2%)	3 (5%)	4 (7%)
Eosinophilic focus	4 (7%)	7 (12%)	14 (23%)
Fatty change, diffuse	5 (8%)	6 (10%)	
Fatty change, focal	9 (15%)	19 (32%)	19 (32%)
Fibrosis	1 (2%)		
Hemorrhage			1 (2%)
Hepatodiaphragmatic nodule	4 (7%)	5 (8%)	2 (3%)
Infarct	1 (2%)	1 (2%)	
Inflammation, granulomatous		16 (27%)	14 (23%)
Mixed cell focus	1 (2%)	1 (2%)	8 (13%)
Necrosis	2 (3%)	1 (2%)	5 (8%)
Pigmentation	1 (2%)	39 (66%)	41 (68%)
Thrombus			1 (2%)
Bile duct, hyperplasia	30 (50%)	15 (25%)	11 (18%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Alimentary System (continued)			
Mesentery	(6)	(4)	(5)
Fibrosis		1 (25%)	
Artery, necrosis, fibrinoid	1 (17%)		1 (20%)
Fat, hemorrhage			1 (20%)
Fat, inflammation, chronic active	1 (17%)	2 (50%)	
Fat, mineralization	1 (17%)		
Fat, necrosis	3 (50%)	1 (25%)	3 (60%)
Pancreas	(59)	(56)	(59)
Hyperplasia	1 (2%)		1 (2%)
Acinus, focal cellular change	1 (2%)		
Acinus, hyperplasia	14 (24%)	20 (36%)	16 (27%)
Artery, inflammation, chronic active	3 (5%)	1 (2%)	
Artery, necrosis, fibrinoid			1 (2%)
Salivary glands	(60)	(59)	(59)
Necrosis		1 (2%)	
Stomach	(59)	(59)	(60)
Artery, necrosis, fibrinoid			1 (2%)
Fat, proliferation		2 (3%)	14 (23%)
Stomach, forestomach	(57)	(59)	(59)
Acanthosis	8 (14%)	25 (42%)	26 (44%)
Fibrosis		1 (2%)	
Hemorrhage	1 (2%)		
Hyperkeratosis	5 (9%)	13 (22%)	17 (29%)
Inflammation, chronic		1 (2%)	
Inflammation, chronic active	1 (2%)	2 (3%)	1 (2%)
Necrosis	2 (4%)		1 (2%)
Pigmentation			1 (2%)
Stomach, glandular	(58)	(59)	(58)
Erosion	1 (2%)	9 (15%)	13 (22%)
Fibrosis		30 (51%)	37 (64%)
Hemorrhage		2 (3%)	5 (9%)
Hyperplasia		10 (17%)	24 (41%)
Hyperplasia, adenomatous		2 (3%)	
Inflammation, acute		9 (15%)	10 (17%)
Metaplasia		26 (44%)	36 (62%)
Mineralization		2 (3%)	1 (2%)
Pigmentation		8 (14%)	19 (33%)
Cardiovascular System			
Heart	(60)	(60)	(60)
Bacterium			1 (2%)
Cardiomyopathy	50 (83%)	52 (87%)	43 (72%)
Mineralization		1 (2%)	
Pigmentation			2 (3%)
Thrombus		1 (2%)	
Artery, necrosis, fibrinoid	1 (2%)		1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Endocrine System			
Adrenal gland	(60)	(60)	(60)
Infarct			1 (2%)
Adrenal gland, cortex	(60)	(60)	(60)
Hyperplasia	2 (3%)	3 (5%)	3 (5%)
Adrenal gland, medulla	(60)	(60)	(59)
Hyperplasia	25 (42%)	18 (30%)	13 (22%)
Islets, pancreatic	(58)	(56)	(59)
Hyperplasia	1 (2%)	2 (4%)	1 (2%)
Parathyroid gland	(56)	(54)	(51)
Hyperplasia	1 (2%)	1 (2%)	
Pituitary gland	(56)	(59)	(56)
Cyst	1 (2%)		
Pars distalis, angiectasis	18 (32%)	15 (25%)	11 (20%)
Pars distalis, cyst	1 (2%)	1 (2%)	1 (2%)
Pars distalis, hyperplasia	14 (25%)	11 (19%)	13 (23%)
Pars distalis, pigmentation	1 (2%)		
Pars intermedia, cyst			1 (2%)
Pars intermedia, hyperplasia		1 (2%)	
Rathke's cleft, cyst		1 (2%)	
Thyroid gland	(60)	(55)	(58)
C-cell, hyperplasia	6 (10%)	5 (9%)	10 (17%)
Follicular cell, cyst	1 (2%)	1 (2%)	
General Body System			
None			
Genital System			
Epididymis	(60)	(58)	(60)
Spermatocele	1 (2%)		
Preputial gland	(56)	(59)	(56)
Inflammation, chronic active	2 (4%)	1 (2%)	
Necrosis	4 (7%)		2 (4%)
Prostate	(59)	(57)	(60)
Abscess	1 (2%)		
Epithelium, hyperplasia	12 (20%)	4 (7%)	7 (12%)
Seminal vesicle	(60)	(59)	(60)
Epithelium, hyperplasia			1 (2%)
Testes	(60)	(60)	(60)
Atrophy		1 (2%)	
Infarct			1 (2%)
Interstitial cell, hyperplasia	26 (43%)	26 (43%)	32 (53%)
Seminiferous tubule, atrophy	29 (48%)	34 (57%)	36 (60%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Hematopoietic System			
Bone marrow	(59)	(58)	(60)
Pigmentation		20 (34%)	37 (62%)
Lymph node	(60)	(59)	(60)
Axillary, angiectasis			1 (2%)
Axillary, infiltration cellular, histiocytic			1 (2%)
Lumbar, infiltration cellular, histiocytic			1 (2%)
Lumbar, pigmentation			1 (2%)
Mediastinal, angiectasis			1 (2%)
Mediastinal, degeneration, cystic	1 (2%)		
Mediastinal, hematopoietic cell proliferation			1 (2%)
Mediastinal, infiltration cellular, histiocytic	8 (13%)	5 (8%)	30 (50%)
Mediastinal, pigmentation	6 (10%)	20 (34%)	25 (42%)
Pancreatic, infiltration cellular, plasma cell	1 (2%)		
Pancreatic, infiltration cellular, histiocytic	3 (5%)	6 (10%)	5 (8%)
Pancreatic, pigmentation		7 (12%)	2 (3%)
Renal, angiectasis		1 (2%)	
Renal, infiltration cellular, histiocytic		1 (2%)	1 (2%)
Renal, pigmentation		1 (2%)	1 (2%)
Lymph node, mandibular	(58)	(56)	(56)
Bacterium			1 (2%)
Degeneration	1 (2%)		
Degeneration, cystic	5 (9%)	1 (2%)	3 (5%)
Infiltration cellular, plasma cell	1 (2%)	1 (2%)	
Infiltration cellular, histiocytic	2 (3%)	3 (5%)	20 (36%)
Pigmentation	2 (3%)	28 (50%)	17 (30%)
Lymph node, mesenteric	(59)	(54)	(58)
Bacterium			1 (2%)
Infiltration cellular, mast cell	1 (2%)	2 (4%)	3 (5%)
Infiltration cellular, histiocytic	26 (44%)	40 (74%)	45 (78%)
Mineralization			3 (5%)
Pigmentation	9 (15%)	42 (78%)	44 (76%)
Spleen	(58)	(56)	(59)
Angiectasis		1 (2%)	
Depletion lymphoid	2 (3%)	5 (9%)	6 (10%)
Fibrosis	3 (5%)	3 (5%)	2 (3%)
Hematopoietic cell proliferation	24 (41%)	35 (63%)	28 (47%)
Hemorrhage	1 (2%)		2 (3%)
Infiltration cellular, histiocytic	15 (26%)	24 (43%)	33 (56%)
Inflammation, granulomatous			1 (2%)
Necrosis			1 (2%)
Pigmentation		33 (59%)	32 (54%)
Pigmentation, hemosiderin	9 (16%)	35 (63%)	37 (63%)
Thrombus			1 (2%)
Capsule, cyst		1 (2%)	
Thymus	(55)	(50)	(52)
Cyst	1 (2%)		
Depletion lymphoid		5 (10%)	7 (13%)
Ectopic parathyroid gland			1 (2%)
Hemorrhage		1 (2%)	
Pigmentation		25 (50%)	31 (60%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Integumentary System			
Mammary gland	(42)	(36)	(41)
Galactocele	4 (10%)	1 (3%)	1 (2%)
Skin	(58)	(60)	(59)
Cyst epithelial inclusion	1 (2%)		
Hemorrhage			1 (2%)
Hyperkeratosis		1 (2%)	1 (2%)
Hyperplasia, squamous	1 (2%)		
Inflammation, chronic active	1 (2%)		
Metaplasia, osseous		1 (2%)	
Necrosis			1 (2%)
Musculoskeletal System			
Bone	(60)	(60)	(60)
Costochondral junction, fibrosis		1 (2%)	
Costochondral junction, inflammation, chronic active		1 (2%)	
Nervous System			
Brain	(60)	(59)	(60)
Bacterium			1 (2%)
Metaplasia, osseous			1 (2%)
Respiratory System			
Lung	(60)	(60)	(60)
Atelectasis		10 (17%)	14 (23%)
Bacterium			1 (2%)
Congestion	6 (10%)	5 (8%)	6 (10%)
Edema		9 (15%)	11 (18%)
Fibrosis		1 (2%)	
Hemorrhage	4 (7%)	3 (5%)	8 (13%)
Infiltration cellular, histiocytic	9 (15%)	46 (77%)	49 (82%)
Inflammation, acute	1 (2%)	13 (22%)	10 (17%)
Inflammation, granulomatous		10 (17%)	20 (33%)
Mineralization		3 (5%)	
Necrosis			1 (2%)
Pigmentation		34 (57%)	50 (83%)
Pigmentation, cholesterol		3 (5%)	5 (8%)
Alveolar epithelium, hyperplasia	4 (7%)	6 (10%)	5 (8%)
Artery, mediastinum, necrosis, fibrinoid			1 (2%)
Bronchiole, hyperplasia		1 (2%)	
Bronchiole, epithelium, metaplasia, squamous			1 (2%)
Bronchus, inflammation, chronic			1 (2%)
Nose	(59)	(60)	(56)
Erosion			1 (2%)
Fungus	1 (2%)	6 (10%)	8 (14%)
Inflammation, acute	8 (14%)	18 (30%)	22 (39%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Respiratory System (continued)			
Trachea	(60)	(58)	(60)
Erosion		1 (2%)	1 (2%)
Inflammation, acute		1 (2%)	3 (5%)
Metaplasia, squamous			3 (5%)
Pigmentation			1 (2%)
Special Senses System			
Ear	(1)	(3)	(1)
Canal, inflammation, acute		2 (67%)	
Eye	(6)	(13)	(9)
Hemorrhage		1 (8%)	
Inflammation, acute		1 (8%)	
Lens, cataract	1 (17%)	6 (46%)	1 (11%)
Retina, atrophy		1 (8%)	1 (11%)
Harderian gland	(6)	(10)	(7)
Inflammation, chronic active			1 (14%)
Pigmentation		1 (10%)	
Urinary System			
Kidney	(60)	(60)	(60)
Abscess			1 (2%)
Bacterium			1 (2%)
Cyst			1 (2%)
Nephropathy	56 (93%)	39 (65%)	34 (57%)
Pigmentation		2 (3%)	
Artery, necrosis, fibrinoid	1 (2%)		
Cortex, mineralization	1 (2%)	3 (5%)	1 (2%)
Papilla, mineralization	6 (10%)	15 (25%)	10 (17%)
Renal tubule, hyperplasia	1 (2%)	1 (2%)	
Renal tubule, pigmentation	24 (40%)	20 (33%)	29 (48%)
Transitional epithelium, hyperplasia	1 (2%)		
Urinary bladder	(60)	(55)	(56)
Calculus gross observation		1 (2%)	1 (2%)
Calculus micro observation only		2 (4%)	2 (4%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF TITANOCENE DICHLORIDE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Disposition Summary			
Animals initially in study	70	71	70
15-month interim evaluation	10	10	10
Early deaths			
Moribund	19	25	21
Dead	3	5	7
Accident	1	1	
Moribund sacrifice			1
Survivors			
Terminal sacrifice	36	30	31
Moribund	1		
Animals examined microscopically	60	61	60
Alimentary System			
Intestine small, duodenum	(59)	(57)	(56)
Leiomyosarcoma			1 (2%)
Liver	(60)	(61)	(60)
Hepatocellular adenoma			2 (3%)
Neoplastic nodule		1 (2%)	
Mesentery	(3)	(9)	(10)
Pancreas	(60)	(61)	(59)
Acinus, adenoma	1 (2%)	1 (2%)	1 (2%)
Pharynx			(1)
Palate, papilloma squamous			1 (100%)
Salivary glands	(60)	(61)	(60)
Stomach, forestomach	(60)	(60)	(60)
Papilloma squamous		1 (2%)	2 (3%)
Stomach, glandular	(60)	(60)	(60)
Tooth	(1)		
Pulp, fibroma	1 (100%)		
Cardiovascular System			
Heart	(60)	(61)	(60)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		
Endocrine System			
Adrenal gland, cortex	(59)	(61)	(60)
Adrenal gland, medulla	(58)	(60)	(59)
Pheochromocytoma malignant	1 (2%)		
Pheochromocytoma benign	6 (10%)	6 (10%)	4 (7%)
Bilateral, pheochromocytoma benign		1 (2%)	2 (3%)
Islets, pancreatic	(59)	(61)	(59)
Adenoma	2 (3%)		
Adenoma, multiple		1 (2%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Endocrine System (continued)			
Pituitary gland	(59)	(59)	(60)
Pars distalis, adenoma	26 (44%)	22 (37%)	16 (27%)
Pars distalis, adenoma, multiple	3 (5%)	1 (2%)	
Thyroid gland	(60)	(57)	(59)
Bilateral, c-cell, adenoma		1 (2%)	
Bilateral, c-cell, carcinoma		2 (4%)	
C-cell, adenoma	9 (15%)	7 (12%)	7 (12%)
C-cell, carcinoma	1 (2%)	1 (2%)	
Follicular cell, adenoma, multiple			1 (2%)
Follicular cell, carcinoma	1 (2%)		
General Body System			
Tissue NOS			(1)
Sarcoma			1 (100%)
Genital System			
Clitoral gland	(56)	(55)	(56)
Adenoma	9 (16%)	8 (15%)	2 (4%)
Carcinoma	1 (2%)	1 (2%)	
Bilateral, adenoma	3 (5%)		
Ovary	(58)	(61)	(60)
Uterus	(60)	(61)	(60)
Adenoma	1 (2%)		
Polyp stromal	11 (18%)	10 (16%)	14 (23%)
Bilateral, polyp stromal			2 (3%)
Cervix, sarcoma			1 (2%)
Hematopoietic System			
Blood	(2)		
Bone marrow	(59)	(61)	(59)
Lymph node	(60)	(61)	(60)
Lymph node, mandibular	(56)	(59)	(60)
Lymph node, mesenteric	(60)	(61)	(60)
Spleen	(60)	(60)	(60)
Sarcoma		1 (2%)	1 (2%)
Thymus	(55)	(56)	(58)
Epithelial cell, thymoma benign	1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Integumentary System			
Mammary gland	(56)	(56)	(49)
Adenocarcinoma	2 (4%)	1 (2%)	
Adenocarcinoma, multiple	1 (2%)		
Adenoma	1 (2%)	1 (2%)	
Fibroadenoma	17 (30%)	17 (30%)	15 (31%)
Fibroadenoma, multiple	9 (16%)	7 (13%)	3 (6%)
Skin	(60)	(61)	(58)
Basal cell carcinoma	1 (2%)		
Subcutaneous tissue, fibroma		1 (2%)	
Subcutaneous tissue, fibrosarcoma	1 (2%)		
Subcutaneous tissue, sarcoma		1 (2%)	1 (2%)
Musculoskeletal System			
None			
Nervous System			
Brain	(60)	(61)	(60)
Astrocytoma malignant	1 (2%)		
Granular cell tumor benign	1 (2%)		1 (2%)
Respiratory System			
Lung	(60)	(61)	(60)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)		
Carcinoma, metastatic, thyroid gland	1 (2%)	1 (2%)	
Carcinoma, metastatic, Zymbal's gland			1 (2%)
Osteosarcoma, metastatic, uncertain primary site	1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal gland	1 (2%)		
Pleura, alveolar/bronchiolar carcinoma, metastatic	1 (2%)		
Special Senses System			
Eye	(5)	(11)	(18)
Zymbal's gland			(1)
Carcinoma			1 (100%)
Urinary System			
Kidney	(60)	(61)	(60)
Renal tubule, adenoma		1 (2%)	
Urinary bladder	(60)	(58)	(60)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Systemic Lesions			
Multiple organs[a]	(60)	(61)	(60)
Leukemia mononuclear	21 (35%)	20 (33%)	15 (25%)
Lymphoma malignant lymphocytic	1 (2%)		
Tumor Summary			
Total animals with primary neoplasms[b]	55	53	46
Total primary neoplasms	135	115	96
Total animals with benign neoplasms	46	50	40
Total benign neoplasms	102	88	75
Total animals with malignant neoplasms	29	25	18
Total malignant neoplasms	33	27	21
Total animals with secondary neoplasms[c]	4	1	1
Total secondary neoplasms	5	1	1
Total animals with malignant neoplasms uncertain primary site	1		

[a] The number in parentheses is the number of animals with any tissue examined microscopically.
[b] Primary tumors: all tumors except metastatic tumors
[c] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control

Number of Days on Study	3 0 4	3 4 2	3 8 4	4 1 3	5 4 9	5 9 8	6 1 1	6 1 5	6 2 2	6 2 4	6 2 4	6 3 9	6 5 1	6 5 2	6 8 8	6 9 3	6 9 4	6 9 5	7 0 0	7 0 2	7 0 7	7 0 9	7 1 5	7 4 3	7 4 3
Carcass ID Number	5 0 5	5 4 5	5 2 5	5 3 5	5 7 5	6 3 5	5 8 4	6 1 4	5 3 3	6 1 3	6 3 4	6 2 4	6 6 3	5 8 3	5 8 2	5 9 5	5 9 5	4 1 1	5 4 3	5 0 5	5 1 4	6 4 4	5 8 1	6 1 2	4 9 1	4 9 2
Alimentary System																										
Esophagus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery																			+							
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																										
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																										
Pulp, fibroma																										
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																							X			
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Pheochromocytoma malignant																										
Pheochromocytoma benign																				X				X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Parathyroid gland	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Pars distalis, adenoma					X		X				X				X	X	X	X		X			X			
Pars distalis, adenoma, multiple																										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma									X											X						
C-cell, carcinoma						X																				
Follicular cell, carcinoma																										

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 5	7 4 9	7 4 9	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0
Carcass ID Number	4 9 4	5 5 4	5 9 4	5 0 2	5 2 3	5 6 2	6 0 2	6 4 3	5 6 1	4 9 3	5 0 4	5 0 1	5 1 2	5 1 4	5 2 1	5 2 2	5 3 1	5 3 2	5 5 1	5 5 2	5 5 3	5 7 3	5 9 1	5 9 2	5 9 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Intestine large, colon		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery									+																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth																									+
Pulp, fibroma																									X
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung																									
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																					X				
Pheochromocytoma benign											X												X		
Islets, pancreatic	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma														X											
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X		X	X				X	X	X	X	X	X	X			X		X	X		X
Pars distalis, adenoma, multiple										X															
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma			X		X	X								X									X		
C-cell, carcinoma																									
Follicular cell, carcinoma						X																			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	
Carcass ID Number	6 0 3	6 0 5	6 1 1	6 2 1	6 2 2	6 2 3	6 3 2	6 3 3	6 4 1	6 4 2	**Total Tissues/ Tumors**
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	58
Intestine large	+	+	+	+	+	+	+	+	+	+	59
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	56
Intestine large, colon	+	+	+	M	+	+	+	+	+	+	57
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	58
Intestine small	+	+	+	+	+	+	+	+	+	+	60
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	59
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	58
Liver	+	+	+	+	+	+	+	+	+	+	60
Mesentery								+			3
Pancreas	+	+	+	+	+	+	+	+	+	+	60
Acinus, adenoma					X						1
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	60
Tooth											1
Pulp, fibroma											1
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar carcinoma, metastatic, lung											1
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	59
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	59
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	58
Pheochromocytoma malignant											1
Pheochromocytoma benign						X			X		6
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	59
Adenoma	X										2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	55
Pituitary gland	+	+	+	+	+	+	+	+	+	+	59
Pars distalis, adenoma	X								X		26
Pars distalis, adenoma, multiple		X	X								3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	60
C-cell, adenoma			X						X		9
C-cell, carcinoma											1
Follicular cell, carcinoma											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	3 0 4	3 4 2	3 8 4	4 1 3	5 4 9	5 9 8	6 1 1	6 1 5	6 2 2	6 2 4	6 2 4	6 3 9	6 5 1	6 5 2	6 8 8	6 9 3	6 9 4	6 9 5	7 0 0	7 0 2	7 0 7	7 0 9	7 1 5	7 4 3	7 4 3
Carcass ID Number	5 0 5	5 4 5	5 2 5	5 3 5	5 7 5	6 3 5	5 8 4	6 1 4	5 3 3	6 1 3	6 3 4	6 2 4	5 6 3	5 8 3	5 8 2	5 9 5	4 9 5	5 4 1	5 0 3	5 1 5	6 4 4	5 8 1	6 1 2	4 9 1	4 9 2
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Adenoma							X																		
Carcinoma																									
Bilateral, adenoma			X																						
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Polyp stromal			X			X									X				X						
Hematopoietic System																									
Blood										+		+													
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+
Epithelial cell, thymoma benign											X														
Integumentary System																									
Mammary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Adenocarcinoma		X																							
Adenocarcinoma, multiple																									
Adenoma																									
Fibroadenoma												X	X				X	X							X
Fibroadenoma, multiple																			X						
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Subcutaneous tissue, fibrosarcoma															X										
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Granular cell tumor benign															X										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 5	7 4 9	7 4 9	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0
Carcass ID Number	4 9 4	5 5 4	5 9 4	5 0 2	5 2 3	5 6 2	6 0 2	6 4 3	5 6 1	4 9 3	5 0 4	5 0 1	5 1 2	5 1 4	5 2 1	5 2 2	5 3 1	5 3 2	5 5 1	5 5 2	5 5 3	5 7 3	5 9 1	5 9 2	5 9 3
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma	X	X				X						X								X		X		X	X
Carcinoma																									
Bilateral, adenoma													X	X											
Ovary		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X														
Polyp stromal											X		X	X	X					X					
Hematopoietic System																									
Blood																									
Bone marrow		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Epithelial cell, thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenocarcinoma																									
Adenocarcinoma, multiple														X											
Adenoma							X														X		X		X
Fibroadenoma			X				X				X		X	X	X		X			X				X	
Fibroadenoma, multiple				X		X																			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma												X													
Subcutaneous tissue, fibrosarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Granular cell tumor benign																							X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	
Carcass ID Number	6 0 3	6 0 5	6 1 1	6 2 1	6 2 2	6 2 3	6 3 2	6 3 3	6 4 1	6 4 2	Total Tissues/ Tumors
General Body System											
None											
Genital System											
Clitoral gland	M	+	M	+	+	+	+	+	+	+	56
Adenoma											9
Carcinoma									X		1
Bilateral, adenoma											3
Ovary	+	+	+	+	+	+	+	+	+	+	58
Uterus	+	+	+	+	+	+	+	+	+	+	60
Adenoma											1
Polyp stromal		X					X				11
Hematopoietic System											
Blood											2
Bone marrow	+	+	+	+	+	+	+	+	+	+	59
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	56
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	60
Spleen	+	+	+	+	+	+	+	+	+	+	60
Thymus	+	+	+	+	+	+	+	+	+	+	55
Epithelial cell, thymoma benign											1
Integumentary System											
Mammary gland	+	+	+	+	+	+	+	+	+	M	56
Adenocarcinoma			X								2
Adenocarcinoma, multiple											1
Adenoma											1
Fibroadenoma			X			X		X			17
Fibroadenoma, multiple	X				X				X		9
Skin	+	+	+	+	+	+	+	+	+	+	60
Basal cell carcinoma											1
Subcutaneous tissue, fibrosarcoma											1
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Astrocytoma malignant		X									1
Granular cell tumor benign											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	3 0 4	3 4 2	3 8 4	4 1 3	5 4 9	5 9 8	6 1 1	6 1 5	6 2 2	6 2 4	6 2 4	6 3 9	6 5 1	6 5 2	6 8 8	6 9 3	6 9 4	6 9 5	7 0 0	7 0 2	7 0 7	7 0 9	7 1 5	7 4 3	7 4 3
Carcass ID Number	5 0 5	5 4 5	5 2 5	5 3 5	5 7 5	6 3 5	5 8 4	6 1 4	5 3 3	6 1 3	6 3 4	6 2 4	5 6 3	5 8 3	5 8 2	5 9 5	4 9 5	5 4 1	5 0 3	5 1 5	6 4 4	5 8 1	6 1 2	4 9 1	4 9 2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, thyroid gland						X																			
Osteosarcoma, metastatic, uncertain primary site																									
Pheochromocytoma malignant, metastatic, adrenal gland																									
Pleura, alveolar/bronchiolar carcinoma, metastatic																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye												+									+		+		
Harderian gland												+									+		+		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X		X	X	X		X	X	X		X					X		X		
Lymphoma malignant lymphocytic												X													

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 5	7 4 9	7 4 9	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0
Carcass ID Number	4 9 4	5 5 4	5 9 4	5 0 2	5 2 3	5 6 2	6 0 2	6 4 3	5 6 1	4 9 3	5 0 4	5 0 1	5 1 2	5 1 4	5 2 1	5 2 2	5 3 1	5 3 2	5 5 1	5 5 2	5 5 3	5 7 3	5 9 1	5 9 2	5 9 3
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Carcinoma, metastatic, thyroid gland																									
Osteosarcoma, metastatic, uncertain primary site												X													
Pheochromocytoma malignant, metastatic, adrenal gland																					X				
Pleura, alveolar/bronchiolar carcinoma, metastatic																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye									+																
Harderian gland																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X			X					X						X	X		X	X	X					
Lymphoma malignant lymphocytic																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: Vehicle Control (continued)

Number of Days on Study	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	7 5 0	
Carcass ID Number	6 0 3	6 0 5	6 1 1	6 1 1	6 2 2	6 2 3	6 3 2	6 3 3	6 4 1	6 4 2	**Total Tissues/ Tumors**
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma			X								1
Alveolar/bronchiolar carcinoma										X	1
Carcinoma, metastatic, thyroid gland											1
Osteosarcoma, metastatic, uncertain primary site											1
Pheochromocytoma malignant, metastatic, adrenal gland											1
Pleura, alveolar/bronchiolar carcinoma, metastatic											1
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Eye			+								5
Harderian gland			+								4
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Urinary bladder	+	+	+	+	+	+	+	+	+	+	60
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear	X		X						X		21
Lymphoma malignant lymphocytic											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg

Number of Days on Study	1 7 7	3 5 0	4 3 0	4 5 1	4 6 4	5 0 4	5 1 0	5 4 9	5 7 0	5 7 4	5 9 0	5 9 5	6 0 8	6 1 0	6 3 1	6 3 9	6 3 9	6 4 3	6 4 5	6 4 5	6 4 5	6 6 0	6 6 1	6 8 8	6 8 8
Carcass ID Number	6 5 5	7 6 5	7 8 5	7 1 5	7 2 5	7 7 5	7 3 5	6 8 5	7 0 5	7 8 4	6 6 4	7 0 4	7 3 4	7 2 2	7 1 4	7 7 5	6 0 4	7 5 5	6 9 4	7 7 3	7 8 3	7 0 3	7 6 3	7 1 2	7 9 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	+	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	A	+	+	+	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	A	+	+	+	A	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Mesentery						+				+							+			+		+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, multiple																									
Parathyroid gland	M	+	+	+	+	+	+	M	+	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma										X		X	X		X	X		X	X	X					
Pars distalis, adenoma, multiple																									
Thyroid gland	A	+	+	A	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, c-cell, adenoma																									
Bilateral, c-cell, carcinoma																									
C-cell, adenoma															X										X
C-cell, carcinoma																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	6 9 2	6 9 4	7 0 7	7 1 5	7 2 1	7 2 1	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9
Carcass ID Number	7 0 2	7 0 1	6 0 4	7 5 4	6 7 3	7 1 1	6 5 3	7 6 1	7 7 2	7 9 3	7 9 5	6 8 3	7 5 2	7 9 1	8 0 1	8 0 2	6 5 1	6 5 2	6 6 1	6 6 2	6 6 3	6 7 1	6 7 2	6 8 1	6 9 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Mesentery															+										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma							X																		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																		X							
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign					X	X					X	X												X	
Bilateral, pheochromocytoma benign										X															
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, multiple																									
Parathyroid gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X					X			X		X	X	X	X	X	X			
Pars distalis, adenoma, multiple									X																
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, c-cell, adenoma																									
Bilateral, c-cell, carcinoma																									
C-cell, adenoma				X	X					X		X		X											
C-cell, carcinoma																							X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	
Carcass ID Number	6 9 3	7 2 1	7 3 1	7 3 2	7 4 1	7 4 3	7 4 4	7 8 1	7 8 2	7 9 2	8 0 3	Total Tissues/ Tumors
Alimentary System												
Esophagus	+	+	+	+	+	+	+	+	+	+	+	61
Intestine large	+	+	+	+	+	+	+	+	+	+	+	60
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	59
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	59
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	59
Intestine small	+	+	+	+	+	+	+	+	+	+	+	57
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	57
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	56
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	56
Liver	+	+	+	+	+	+	+	+	+	+	+	61
Neoplastic nodule										X		1
Mesentery			+						+			9
Pancreas	+	+	+	+	+	+	+	+	+	+	+	61
Acinus, adenoma												1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	61
Stomach	+	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	60
Papilloma squamous												1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	60
Cardiovascular System												
Heart	+	+	+	+	+	+	+	+	+	+	+	61
Endocrine System												
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	61
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	61
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	60
Pheochromocytoma benign					X							6
Bilateral, pheochromocytoma benign												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	61
Adenoma, multiple								X				1
Parathyroid gland	M	M	+	+	+	+	+	+	+	+	+	53
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	59
Pars distalis, adenoma	X	X		X			X				X	22
Pars distalis, adenoma, multiple												1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	57
Bilateral, c-cell, adenoma				X								1
Bilateral, c-cell, carcinoma	X	X										2
C-cell, adenoma												7
C-cell, carcinoma												1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	1 7 7	3 5 0	4 3 0	4 5 1	4 6 4	5 0 4	5 1 0	5 4 9	5 7 0	5 7 4	5 9 0	5 9 5	6 0 8	6 1 0	6 3 1	6 3 9	6 3 9	6 4 3	6 4 5	6 4 5	6 4 5	6 6 0	6 6 1	6 8 8	6 8 8
Carcass ID Number	6 5 5	7 6 5	7 8 5	7 1 5	7 2 5	7 7 5	7 3 5	6 8 5	7 0 5	7 8 4	7 6 4	7 0 4	7 3 4	7 2 2	7 1 4	6 7 5	8 0 4	7 5 5	6 9 4	7 7 3	7 8 3	7 0 3	7 6 3	7 1 2	7 9 4
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	M	M	+	M
Adenoma							X				X	X													
Carcinoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal									X			X					X				X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	M	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma		X																							
Adenoma										X															
Fibroadenoma						X										X	X			X	X	X			X
Fibroadenoma, multiple															X								X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	6 9 2	6 9 4	7 0 7	7 1 5	7 2 1	7 2 1	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9
Carcass ID Number	7 0 2	7 0 1	6 8 4	7 5 4	6 7 3	7 1 1	6 5 3	7 6 1	7 7 2	7 9 3	7 9 5	6 8 3	7 5 2	7 9 1	8 0 1	8 0 2	6 5 1	6 5 2	6 6 1	6 6 2	6 6 3	6 7 1	6 7 2	6 8 1	6 9 1
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X											X	X			X						
Carcinoma										X															
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal						X							X						X			X			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma								X																	
Thymus	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Adenocarcinoma																									
Adenoma																									
Fibroadenoma		X				X	X					X	X				X	X		X					
Fibroadenoma, multiple										X													X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma			X																						
Subcutaneous tissue, sarcoma			X																						
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	
Carcass ID Number	6 9 3	7 2 1	7 3 1	7 3 2	7 4 1	7 4 3	7 4 4	7 8 1	7 8 2	7 9 2	8 0 3	Total Tissues/ Tumors
General Body System												
None												
Genital System												
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	55
Adenoma									X			8
Carcinoma												1
Ovary	+	+	+	+	+	+	+	+	+	+	+	61
Uterus	+	+	+	+	+	+	+	+	+	+	+	61
Polyp stromal			X		X							10
Hematopoietic System												
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	61
Lymph node	+	+	+	+	+	+	+	+	+	+	+	61
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	59
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	61
Spleen	+	+	+	+	+	+	+	+	+	+	+	60
Sarcoma												1
Thymus	+	+	M	+	+	+	+	+	+	+	+	56
Integumentary System												
Mammary gland	+	+	+	M	+	+	+	+	+	M	+	56
Adenocarcinoma												1
Adenoma												1
Fibroadenoma		X	X									17
Fibroadenoma, multiple					X			X			X	7
Skin	+	+	+	+	+	+	+	+	+	+	+	61
Subcutaneous tissue, fibroma												1
Subcutaneous tissue, sarcoma												1
Musculoskeletal System												
Bone	+	+	+	+	+	+	+	+	+	+	+	61
Nervous System												
Brain	+	+	+	+	+	+	+	+	+	+	+	61

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	1 7 7	3 5 0	4 3 0	4 5 1	4 6 4	5 0 4	5 1 0	5 4 9	5 7 0	5 7 4	5 9 0	5 9 5	6 0 8	6 1 0	6 3 1	6 3 9	6 3 9	6 4 3	6 4 5	6 4 5	6 4 5	6 6 0	6 6 1	6 8 8	6 8 8
Carcass ID Number	6 5 5	7 6 5	7 8 5	7 1 5	7 2 5	7 7 5	7 3 5	6 8 5	7 0 5	7 8 4	6 6 4	7 0 4	7 3 4	7 2 2	7 1 4	6 7 5	8 0 4	7 5 5	6 9 4	7 7 3	7 8 3	7 0 3	7 6 3	7 1 2	7 9 4
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																+									
Eye		+	+						+							+		+			+		+		+
Harderian gland			+													+		+			+		+		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma																									
Urinary bladder	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear									X		X			X				X	X			X			X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	6 9 2	6 9 4	7 0 7	7 1 5	7 2 1	7 2 1	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9
Carcass ID Number	7 0 2	7 0 1	6 8 4	7 5 4	6 7 3	7 1 1	6 5 3	7 6 1	7 7 2	7 9 3	7 9 5	6 8 3	7 5 2	7 9 1	8 0 1	8 0 2	6 5 1	6 5 2	6 6 1	6 6 2	6 6 3	6 7 1	6 7 2	6 8 1	6 9 1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma									X																
Carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye					+									+											
Harderian gland					+									+											
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma	X																								
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X	X	X	X						X				X	X	X					X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 25 mg/kg (continued)

Number of Days on Study	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	
Carcass ID Number	6 9 3	7 2 1	7 3 1	7 3 2	7 4 1	7 4 3	7 4 4	7 8 1	7 8 2	7 9 2	8 0 3	Total Tissues/ Tumors
Respiratory System												
Lung	+	+	+	+	+	+	+	+	+	+	+	61
Alveolar/bronchiolar adenoma												1
Carcinoma, metastatic, thyroid gland		X										1
Nose	+	+	+	+	+	+	+	+	+	+	+	61
Trachea	+	+	+	+	+	+	+	+	+	+	+	59
Special Senses System												
Ear												1
Eye										+		11
Harderian gland										+		8
Urinary System												
Kidney	+	+	+	+	+	+	+	+	+	+	+	61
Renal tubule, adenoma												1
Urinary bladder	+	+		+	+	+	+	+	+	+	+	58
Systemic Lesions												61
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	61
Leukemia mononuclear								X	X			20

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg

Number of Days on Study	2 3 2	2 3 4	2 4 7	3 0 7	3 3 8	3 4 4	3 4 5	4 1 5	4 5 7	4 5 7	4 7 1	4 8 5	4 9 3	4 9 3	4 9 6	5 2 1	5 7 5	6 1 7	6 3 9	6 4 3	6 5 3	6 6 5	6 6 5	6 7 3	6 7 4
Carcass ID Number	8 6 5	8 2 5	9 6 5	8 5 5	8 2 4	9 6 4	9 0 5	9 3 5	8 3 4	8 5 4	9 5 5	9 5 4	8 4 5	8 7 5	9 1 5	8 8 5	8 5 3	8 6 4	9 3 3	9 3 2	8 8 4	8 1 4	8 7 4	8 8 3	8 9 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	A	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	A	+	+	+	A	+	+	+	+
Leiomyosarcoma																									
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	A	+	+	+	A	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery										+															
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Acinus, adenoma																									
Pharynx																									
Palate, papilloma squamous																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Bilateral, pheochromocytoma benign																									X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Parathyroid gland	M	+	M	M	+	M	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																	X		X	X				X	
Thyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																					X				
Follicular cell, adenoma, multiple																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	6 8 3	6 9 3	6 9 4	7 4 2	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8
Carcass ID Number	8 8 2	9 0 4	9 3 1	8 1 3	8 7 1	9 1 1	9 1 4	9 2 3	9 5 2	8 2 1	8 8 1	8 9 1	9 1 3	9 4 5	8 1 1	8 1 2	8 3 1	8 3 2	8 4 1	8 4 2	8 5 1	8 5 2	8 6 3	9 0 2	9 0 3
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma								X																
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X																			X
Mesentery		+					+		+				+		+					+		+		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma								X																
Pharynx													+											
Palate, papilloma squamous													X											
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																X					X			
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign		X					X	X						X										
Bilateral, pheochromocytoma benign																								
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X	X		X						X	X	X						X	X					X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma											X	X													X
Follicular cell, adenoma, multiple		X																							

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	7 4 8	7 4 8	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	
Carcass ID Number	9 4 4	9 5 1	8 7 2	8 7 3	8 9 2	8 9 3	9 1 2	9 2 1	9 4 2	9 4 3	Total Tissues/ Tumors
Alimentary System											
Esophagus	+	+	+	+	+	+	+	+	+	+	59
Intestine large	+	+	+	+	+	+	+	+	+	+	59
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	57
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	59
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	58
Intestine small	+	+	+	+	+	+	+	+	+	+	59
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	56
Leiomyosarcoma											1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	58
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	56
Liver	+	+	+	+	+	+	+	+	+	+	60
Hepatocellular adenoma											2
Mesentery			+								10
Pancreas	+	+	+	+	+	+	+	+	+	+	59
Acinus, adenoma											1
Pharynx											1
Palate, papilloma squamous											1
Salivary glands	+	+	+	+	+	+	+	+	+	+	60
Stomach	+	+	+	+	+	+	+	+	+	+	60
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	60
Papilloma squamous											2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	60
Cardiovascular System											
Heart	+	+	+	+	+	+	+	+	+	+	60
Endocrine System											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	60
Adrenal gland, medulla	M	+	+	+	+	+	+	+	+	+	59
Pheochromocytoma benign											4
Bilateral, pheochromocytoma benign										X	2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	59
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	51
Pituitary gland	+	+	+	+	+	+	+	+	+	+	60
Pars distalis, adenoma		X			X	X					16
Thyroid gland	+	+	+	+	+	+	+	+	+	+	59
C-cell, adenoma	X					X	X				7
Follicular cell, adenoma, multiple											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	223	233	244	307	338	344	345	411	557	577	717	785	899	993	963	521	755	617	633	649	655	665	675	677	674
Carcass ID Number	865	825	965	855	824	946	005	935	834	545	555	544	545	475	515	185	535	644	633	532	844	811	744	838	894
General Body System																									
Tissue NOS																	+								
Sarcoma																	X								
Genital System																									
Clitoral gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	M	+	+
Adenoma												X													
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal													X						X	X	X				
Bilateral, polyp stromal																									
Cervix, sarcoma																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Sarcoma																						X			
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Integumentary System																									
Mammary gland	M	+	+	M	+	+	+	+	M	M	+	M	+	+	+	+	M	+	+	+	+	+	+	+	
Fibroadenoma														X				X							
Fibroadenoma, multiple																						X			
Skin	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Subcutaneous tissue, sarcoma										X															
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Granular cell tumor benign																									
Spinal cord																			+						

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	6 8 3	6 9 3	6 9 4	7 4 2	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8
Carcass ID Number	8 8 2	9 0 4	9 3 1	8 1 3	8 7 1	9 1 1	9 1 4	9 2 3	9 5 2	8 2 1	8 8 1	8 9 1	9 1 3	9 4 5	8 1 1	8 1 2	8 3 1	8 3 2	8 4 1	8 4 2	8 5 1	8 5 2	8 6 3	9 0 2	9 0 3
General Body System																									
Tissue NOS																									
Sarcoma																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal				X												X			X	X				X	
Bilateral, polyp stromal									X																X
Cervix, sarcoma					X																				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	M	+	M	+	+	+	+	+	+
Fibroadenoma			X		X	X		X							X			X		X			X		X
Fibroadenoma, multiple													X									X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor benign		X																							
Spinal cord																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	7 4 8	7 4 8	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	
Carcass ID Number	9 4 4	9 5 1	8 7 2	8 7 3	8 9 2	8 9 3	9 1 2	9 2 1	9 4 2	9 4 3	**Total Tissues/ Tumors**
General Body System											
Tissue NOS											1
Sarcoma											1
Genital System											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	56
Adenoma						X					2
Ovary	+	+	+	+	+	+	+	+	+	+	60
Uterus	+	+	+	+	+	+	+	+	+	+	60
Polyp stromal	X		X	X		X				X	14
Bilateral, polyp stromal											2
Cervix, sarcoma											1
Hematopoietic System											
Bone marrow	+	+	+	+	+	+	+	+	+	+	59
Lymph node	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	60
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	60
Spleen	+	+	+	+	+	+	+	+	+	+	60
Sarcoma											1
Thymus	+	+	+	+	+	+	+	+	+	+	58
Integumentary System											
Mammary gland	+	+	+	+	+	+	+	M	+	+	49
Fibroadenoma		X			X		X			X	15
Fibroadenoma, multiple											3
Skin	+	M	+	+	+	+	+	+	+	+	58
Subcutaneous tissue, sarcoma											1
Musculoskeletal System											
Bone	+	+	+	+	+	+	+	+	+	+	60
Nervous System											
Brain	+	+	+	+	+	+	+	+	+	+	60
Granular cell tumor benign											1
Spinal cord											1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	2 3 2	2 3 4	2 4 7	3 0 7	3 3 8	3 4 4	3 4 5	4 1 5	4 5 7	4 5 7	4 7 1	4 8 5	4 9 3	4 9 3	4 9 6	5 2 1	5 7 5	6 1 7	6 3 9	6 4 3	6 5 3	6 6 5	6 6 5	6 7 3	6 7 4
Carcass ID Number	8 6 5	8 2 5	9 6 5	8 5 5	8 2 4	9 6 4	9 0 5	9 3 5	8 3 4	8 5 4	9 5 5	9 5 4	8 4 5	8 7 5	9 1 5	8 8 5	8 5 3	8 6 4	9 3 3	9 3 2	8 8 4	8 1 4	8 7 4	8 8 3	8 9 4
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar adenoma, multiple																									
Carcinoma, metastatic, Zymbal's gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear		+							+							+			+						
Eye		+			+							+	+						+			+		+	
Harderian gland					+								+						+			+			
Zymbal's gland																									
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X																X			X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	6 8 3	6 9 3	6 9 4	7 4 2	7 4 3	7 4 3	7 4 3	7 4 3	7 4 3	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 4	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8	7 4 8
Carcass ID Number	8 8 2	9 0 4	9 3 1	8 1 3	8 7 1	9 1 1	9 1 4	9 2 3	9 5 2	8 2 1	8 8 1	8 9 1	9 1 3	9 4 5	8 1 1	8 1 2	8 3 1	8 3 2	8 4 1	8 4 2	8 5 1	8 5 2	8 6 3	9 0 2	9 0 3
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma												X													
Alveolar/bronchiolar adenoma, multiple																									X
Carcinoma, metastatic, Zymbal's gland											X														
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear				+							+														
Eye	+	+		+	+						+			+					+	+	+				
Harderian gland	+	+		+	+						+			+						+	+				
Zymbal's gland											+														
Carcinoma											X														
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X				X			X			X				X							X		X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of Titanocene Dichloride: 50 mg/kg (continued)

Number of Days on Study	7 4 8	7 4 8	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	7 4 9	
Carcass ID Number	9 4 4	9 5 1	8 7 2	8 7 3	8 9 2	8 9 3	9 1 2	9 2 1	9 4 2	9 4 3	Total Tissues/ Tumors
Respiratory System											
Lung	+	+	+	+	+	+	+	+	+	+	60
Alveolar/bronchiolar adenoma											1
Alveolar/bronchiolar adenoma, multiple											1
Carcinoma, metastatic, Zymbal's gland											1
Nose	+	+	+	+	+	+	+	+	+	+	60
Trachea	+	+	+	+	+	+	+	+	+	+	60
Special Senses System											
Ear											6
Eye		+						+			18
Harderian gland		+						+			14
Zymbal's gland											1
Carcinoma											1
Urinary System											
Kidney	+	+	+	+	+	+	+	+	+	+	60
Urinary bladder	+	+	+	+	+	+	+	+	+	+	60
Systemic Lesions											
Multiple organs	+	+	+	+	+	+	+	+	+	+	60
Leukemia mononuclear	X	X						X	X	X	15

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Adrenal Gland (Medulla): Pheochromocytoma Benign			
Overall rates[a]	6/58 (10%)	7/60 (12%)	6/59 (10%)
Adjusted rates[b]	16.0%	21.9%	18.2%
Terminal rates[c]	5/36 (14%)	5/30 (17%)	4/30 (13%)
First incidence (days)	702	721	674
Life table tests[d]	P=0.414	P=0.368	P=0.475
Logistic regression tests[d]	P=0.416	P=0.393	P=0.487
Cochran-Armitage test[d]	P=0.547N		
Fisher exact test[d]		P=0.526	P=0.607N
Adrenal Gland (Medulla): Pheochromocytoma Benign or Malignant			
Overall rates	7/58 (12%)	7/60 (12%)	6/59 (10%)
Adjusted rates	18.7%	21.9%	18.2%
Terminal rates	6/36 (17%)	5/30 (17%)	4/30 (13%)
First incidence (days)	702	721	674
Life table tests	P=0.523	P=0.473	P=0.583
Logistic regression tests	P=0.528	P=0.502	P=0.595
Cochran-Armitage test	P=0.429N		
Fisher exact test		P=0.585N	P=0.487N
Clitoral Gland: Adenoma			
Overall rates	12/56 (21%)	8/55 (15%)	2/56 (4%)
Adjusted rates	31.9%	20.7%	5.2%
Terminal rates	10/34 (29%)	4/30 (13%)	1/31 (3%)
First incidence (days)	384	510	485
Life table tests	P=0.009N	P=0.323N	P=0.009N
Logistic regression tests	P=0.005N	P=0.259N	P=0.007N
Cochran-Armitage test	P=0.004N		
Fisher exact test		P=0.244N	P=0.004N
Clitoral Gland: Adenoma or Carcinoma			
Overall rates	13/56 (23%)	9/55 (16%)	2/56 (4%)
Adjusted rates	34.8%	23.8%	5.2%
Terminal rates	11/34 (32%)	5/30 (17%)	1/31 (3%)
First incidence (days)	384	510	485
Life table tests	P=0.006N	P=0.338N	P=0.005N
Logistic regression tests	P=0.003N	P=0.277N	P=0.004N
Cochran-Armitage test	P=0.002N		
Fisher exact test		P=0.253N	P=0.002N
Mammary Gland: Fibroadenoma			
Overall rates	26/60 (43%)	24/61 (39%)	18/60 (30%)
Adjusted rates	61.3%	56.8%	50.5%
Terminal rates	21/37 (57%)	13/30 (43%)	14/31 (45%)
First incidence (days)	639	504	496
Life table tests	P=0.283N	P=0.387	P=0.285N
Logistic regression tests	P=0.238N	P=0.571N	P=0.280N
Cochran-Armitage test	P=0.079N		
Fisher exact test		P=0.397N	P=0.092N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Mammary Gland: Adenoma or Fibroadenoma			
Overall rates	26/60 (43%)	25/61 (41%)	18/60 (30%)
Adjusted rates	61.3%	57.6%	50.5%
Terminal rates	21/37 (57%)	13/30 (43%)	14/31 (45%)
First incidence (days)	639	504	496
Life table tests	P=0.289N	P=0.323	P=0.285N
Logistic regression tests	P=0.234N	P=0.523	P=0.280N
Cochran-Armitage test	P=0.079N		
Fisher exact test		P=0.469N	P=0.092N
Mammary Gland: Adenocarcinoma			
Overall rates	3/60 (5%)	1/61 (2%)	0/60 (0%)
Adjusted rates	7.0%	1.7%	0.0%
Terminal rates	2/37 (5%)	0/30 (0%)	0/31 (0%)
First incidence (days)	342	350	–[e]
Life table tests	P=0.077N	P=0.341N	P=0.149N
Logistic regression tests	P=0.023N	P=0.227N	P=0.084N
Cochran-Armitage test	P=0.060N		
Fisher exact test		P=0.303N	P=0.122N
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall rates	27/60 (45%)	26/61 (43%)	18/60 (30%)
Adjusted rates	62.0%	58.3%	50.5%
Terminal rates	21/37 (57%)	13/30 (43%)	14/31 (45%)
First incidence (days)	342	350	496
Life table tests	P=0.239N	P=0.329	P=0.228N
Logistic regression tests	P=0.151N	P=0.552N	P=0.198N
Cochran-Armitage test	P=0.056N		
Fisher exact test		P=0.468N	P=0.066N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	29/59 (49%)	23/59 (39%)	16/60 (27%)
Adjusted rates	62.3%	58.0%	42.3%
Terminal rates	20/37 (54%)	15/30 (50%)	10/31 (32%)
First incidence (days)	549	574	575
Life table tests	P=0.079N	P=0.481N	P=0.089N
Logistic regression tests	P=0.032N	P=0.267N	P=0.041N
Cochran-Armitage test	P=0.008N		
Fisher exact test		P=0.177N	P=0.009N
Thyroid Gland (C-Cell): Adenoma			
Overall rates	9/60 (15%)	8/57 (14%)	7/59 (12%)
Adjusted rates	22.4%	22.4%	21.4%
Terminal rates	7/37 (19%)	4/30 (13%)	6/31 (19%)
First incidence (days)	622	631	653
Life table tests	P=0.500N	P=0.546	P=0.548N
Logistic regression tests	P=0.501N	P=0.601N	P=0.558N
Cochran-Armitage test	P=0.358N		
Fisher exact test		P=0.546N	P=0.409N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Thyroid Gland (C-Cell): Carcinoma			
Overall rates	1/60 (2%)	3/57 (5%)	0/59 (0%)
Adjusted rates	1.8%	10.0%	0.0%
Terminal rates	0/37 (0%)	3/30 (10%)	0/31 (0%)
First incidence (days)	598	743 (T)	–
Life table tests	P=0.443N	P=0.243	P=0.549N
Logistic regression tests	P=0.424N	P=0.277	P=0.461N
Cochran-Armitage test	P=0.385N		
Fisher exact test		P=0.290	P=0.504N
Thyroid Gland (C-Cell): Adenoma or Carcinoma			
Overall rates	10/60 (17%)	11/57 (19%)	7/59 (12%)
Adjusted rates	23.8%	31.4%	21.4%
Terminal rates	7/37 (19%)	7/30 (23%)	6/31 (19%)
First incidence (days)	598	631	653
Life table tests	P=0.428N	P=0.326	P=0.455N
Logistic regression tests	P=0.420N	P=0.394	P=0.446N
Cochran-Armitage test	P=0.279N		
Fisher exact test		P=0.448	P=0.314N
Uterus: Stromal Polyp			
Overall rates	11/60 (18%)	10/61 (16%)	16/60 (27%)
Adjusted rates	25.3%	25.8%	43.2%
Terminal rates	7/37 (19%)	5/30 (17%)	11/31 (35%)
First incidence (days)	384	570	493
Life table tests	P=0.072	P=0.525	P=0.087
Logistic regression tests	P=0.086	P=0.504N	P=0.103
Cochran-Armitage test	P=0.154		
Fisher exact test		P=0.483N	P=0.191
All Organs: Mononuclear Cell Leukemia			
Overall rates	21/60 (35%)	20/61 (33%)	15/60 (25%)
Adjusted rates	43.3%	45.5%	41.7%
Terminal rates	11/37 (30%)	7/30 (23%)	11/31 (35%)
First incidence (days)	598	570	344
Life table tests	P=0.372N	P=0.403	P=0.377N
Logistic regression tests	P=0.252N	P=0.534N	P=0.283N
Cochran-Armitage test	P=0.139N		
Fisher exact test		P=0.474N	P=0.160N
All Organs: Benign Tumors			
Overall rates	46/60 (77%)	50/61 (82%)	40/60 (67%)
Adjusted rates	88.2%	94.3%	88.7%
Terminal rates	31/37 (84%)	27/30 (90%)	26/31 (84%)
First incidence (days)	384	504	485
Life table tests	P=0.366	P=0.046	P=0.431
Logistic regression tests	P=0.525	P=0.152	P=0.588
Cochran-Armitage test	P=0.123N		
Fisher exact test		P=0.310	P=0.156N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
All Organs: Malignant Tumors			
Overall rates	30/60 (50%)	25/61 (41%)	18/60 (30%)
Adjusted rates	59.1%	55.7%	45.6%
Terminal rates	17/37 (46%)	11/30 (37%)	11/31 (35%)
First incidence (days)	342	350	344
Life table tests	P=0.136N	P=0.540N	P=0.136N
Logistic regression tests	P=0.037N	P=0.247N	P=0.044N
Cochran-Armitage test	P=0.016N		
Fisher exact test		P=0.208N	P=0.020N
All Organs: Benign or Malignant Tumors			
Overall rates	56/60 (93%)	53/61 (87%)	46/60 (77%)
Adjusted rates	96.5%	96.3%	93.8%
Terminal rates	35/37 (95%)	28/30 (93%)	28/31 (90%)
First incidence (days)	342	350	344
Life table tests	P=0.519N	P=0.226	P=0.524N
Logistic regression tests	P=0.092N	P=0.267N	P=0.119N
Cochran-Armitage test	P=0.007N		
Fisher exact test		P=0.189N	P=0.010N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group.

TABLE B4
Historical Incidence of Neoplasms of the Forestomach in Female F344/N Rats Receiving Corn Oil Vehicle

Study	Incidence in Controls		
	Squamous Papilloma	Squamous Cell Carcinoma	Squamous Papilloma or Squamous Cell Carcinoma
Historical Incidence at EG&G Mason Research Institute[a]			
Bromoform	1/50	0/50	1/50
Hexachloroethane	0/50	0/50	0/50
Phenylbutazone	1/50	0/50	1/50
Probenecid	0/50	0/50	0/50
Diglycidyl resorcinol ether	0/49	0/49	0/49
Diglycidyl resorcinol ether	0/50	0/50	0/50
1,2-dichloropropane	0/50	0/50	0/50
Chlorodibromomethane	0/50	0/50	0/50
N-butyl chloride	2/49	0/49	2/49
Bromodichloromethane	0/50	1/50	1/50
Total	4/498 (0.8%)	1/498 (0.2%)	5/498 (1.0%)
Standard deviation	1.4%	0.6%	1.4%
Range	0%–4%	0%–2%	0%–4%
Overall Historical Incidence[a]			
Total	7/2,748[b] (0.3%)	1/2,748 (0.04%)	8/2,748[b] (0.3%)
Standard deviation	0.8%	0.3%	0.8%
Range	0%–4%	0%–2%	0%–4%

[a] Toxicology Data Management System compilation (data as of 22 December 1989), and Carcinogenesis Bioassay Data System compilation (data as of 6 March 1990)
[b] Includes one papilloma NOS.

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride

	Vehicle Control	25 mg/kg	50 mg/kg
Disposition Summary			
Animals initially in study	70	71	70
15-month interim evaluation	10	10	10
Early deaths			
Moribund	19	25	21
Dead	3	5	7
Accident	1	1	
Moribund sacrifice			1
Survivors			
Terminal sacrifice	36	30	31
Moribund	1		
Animals examined microscopically	60	61	60
Alimentary System			
Intestine large, colon	(57)	(59)	(59)
Erosion		1 (2%)	
Inflammation, acute		1 (2%)	
Parasite	2 (4%)		1 (2%)
Intestine small, duodenum	(59)	(57)	(56)
Hyperplasia, adenomatous		1 (2%)	
Pigmentation		25 (44%)	34 (61%)
Intestine small, ileum	(59)	(56)	(58)
Fibrosis		1 (2%)	
Inflammation, acute		2 (4%)	
Pigmentation		1 (2%)	1 (2%)
Intestine small, jejunum	(58)	(56)	(56)
Pigmentation		1 (2%)	9 (16%)
Liver	(60)	(61)	(60)
Abscess			1 (2%)
Angiectasis		2 (3%)	2 (3%)
Basophilic focus	45 (75%)	45 (74%)	48 (80%)
Clear cell focus	1 (2%)	3 (5%)	10 (17%)
Degeneration, cystic	1 (2%)	1 (2%)	
Eosinophilic focus			2 (3%)
Fatty change, diffuse	11 (18%)	6 (10%)	10 (17%)
Fatty change, focal	15 (25%)	11 (18%)	14 (23%)
Hepatodiaphragmatic nodule	4 (7%)	6 (10%)	10 (17%)
Hyperplasia		1 (2%)	
Inflammation, granulomatous	6 (10%)	24 (39%)	33 (55%)
Mixed cell focus	2 (3%)	5 (8%)	3 (5%)
Necrosis	3 (5%)	6 (10%)	7 (12%)
Pigmentation	3 (5%)	45 (74%)	50 (83%)
Thrombus	1 (2%)		
Bile duct, hyperplasia	17 (28%)	4 (7%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Alimentary System (continued)			
Mesentery	(3)	(9)	(10)
Fat, inflammation, chronic active		2 (22%)	2 (20%)
Fat, necrosis	1 (33%)	7 (78%)	6 (60%)
Pancreas	(60)	(61)	(59)
Fibrosis			1 (2%)
Inflammation, chronic active			1 (2%)
Acinus, atrophy		1 (2%)	
Acinus, hyperplasia	6 (10%)	4 (7%)	8 (14%)
Salivary glands	(60)	(61)	(60)
Inflammation, chronic active			1 (2%)
Stomach	(60)	(60)	(60)
Fat, proliferation		15 (25%)	41 (68%)
Polyp adenomatous, inflammation		1 (2%)	
Stomach, forestomach	(60)	(60)	(60)
Acanthosis	11 (18%)	20 (33%)	27 (45%)
Hyperkeratosis	10 (17%)	23 (38%)	21 (35%)
Inflammation, acute	1 (2%)		4 (7%)
Necrosis	6 (10%)		
Stomach, glandular	(60)	(60)	(60)
Erosion	2 (3%)	11 (18%)	10 (17%)
Fibrosis		39 (65%)	51 (85%)
Hemorrhage		3 (5%)	2 (3%)
Hyperplasia		24 (40%)	23 (38%)
Hyperplasia, adenomatous		1 (2%)	
Inflammation, acute		4 (7%)	5 (8%)
Metaplasia		36 (60%)	51 (85%)
Mineralization		7 (12%)	2 (3%)
Pigmentation		19 (32%)	24 (40%)
Cardiovascular System			
Heart	(60)	(61)	(60)
Bacterium	1 (2%)		
Cardiomyopathy	23 (38%)	23 (38%)	23 (38%)
Mineralization		1 (2%)	
Thrombus		1 (2%)	
Endocrine System			
Adrenal gland, cortex	(59)	(61)	(60)
Degeneration		1 (2%)	
Degeneration, cystic		1 (2%)	
Hemorrhage		1 (2%)	
Hyperplasia	3 (5%)	2 (3%)	1 (2%)
Necrosis		1 (2%)	1 (2%)
Pigmentation, ceroid		1 (2%)	
Adrenal gland, medulla	(58)	(60)	(59)
Hyperplasia	13 (22%)	8 (13%)	6 (10%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Endocrine System (continued)			
Parathyroid gland	(55)	(53)	(51)
Hyperplasia			1 (2%)
Pituitary gland	(59)	(59)	(60)
Cyst	1 (2%)		
Pars distalis, angiectasis	27 (46%)	21 (36%)	23 (38%)
Pars distalis, cyst	4 (7%)	4 (7%)	2 (3%)
Pars distalis, hyperplasia	13 (22%)	18 (31%)	24 (40%)
Pars distalis, pigmentation	1 (2%)		
Pars intermedia, cyst	1 (2%)	1 (2%)	
Pars intermedia, hyperplasia	1 (2%)		
Pars nervosa, cyst		1 (2%)	
Thyroid gland	(60)	(57)	(59)
C-cell, hyperplasia	8 (13%)	17 (30%)	13 (22%)
Follicle, cyst	1 (2%)		
Follicular cell, hyperplasia			2 (3%)
General Body System			
Tissue NOS			(1)
Hemorrhage			1 (100%)
Genital System			
Clitoral gland	(56)	(55)	(56)
Inflammation, acute			1 (2%)
Necrosis	1 (2%)	1 (2%)	
Ovary	(58)	(61)	(60)
Cyst	3 (5%)	6 (10%)	1 (2%)
Degeneration, cystic		1 (2%)	
Hyperplasia, tubular		1 (2%)	
Uterus	(60)	(61)	(60)
Cyst			2 (3%)
Decidual reaction			1 (2%)
Hemorrhage		2 (3%)	3 (5%)
Inflammation, acute			2 (3%)
Necrosis			1 (2%)
Prolapse			1 (2%)
Thrombus			1 (2%)
Cervix, hemorrhage	1 (2%)		
Cervix, inflammation, acute			2 (3%)
Endometrium, hyperplasia		2 (3%)	2 (3%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Hematopoietic System			
Bone marrow	(59)	(61)	(59)
Pigmentation		8 (13%)	26 (44%)
Lymph node	(60)	(61)	(60)
Lumbar, degeneration, cystic			1 (2%)
Mediastinal, angiectasis	1 (2%)		
Mediastinal, congestion			1 (2%)
Mediastinal, hemorrhage		1 (2%)	
Mediastinal, infiltration cellular, plasma cell		1 (2%)	
Mediastinal, infiltration cellular, histiocytic	12 (20%)	21 (34%)	27 (45%)
Mediastinal, pigmentation	1 (2%)	23 (38%)	25 (42%)
Pancreatic, angiectasis		1 (2%)	1 (2%)
Pancreatic, hematopoietic cell proliferation			1 (2%)
Pancreatic, infiltration cellular, histiocytic	3 (5%)	5 (8%)	17 (28%)
Pancreatic, pigmentation		10 (16%)	18 (30%)
Renal, angiectasis			1 (2%)
Renal, infiltration cellular, histiocytic			1 (2%)
Lymph node, mandibular	(56)	(59)	(60)
Infiltration cellular, histiocytic	9 (16%)	6 (10%)	22 (37%)
Pigmentation		34 (58%)	26 (43%)
Lymph node, mesenteric	(60)	(61)	(60)
Angiectasis			2 (3%)
Degeneration, cystic	1 (2%)		
Depletion lymphoid		1 (2%)	
Infiltration cellular, histiocytic	27 (45%)	45 (74%)	47 (78%)
Pigmentation	1 (2%)	47 (77%)	49 (82%)
Spleen	(60)	(60)	(60)
Angiectasis			3 (5%)
Depletion lymphoid	2 (3%)	3 (5%)	2 (3%)
Fibrosis	1 (2%)	1 (2%)	
Hematopoietic cell proliferation	33 (55%)	43 (72%)	43 (72%)
Hemorrhage		1 (2%)	
Infiltration cellular, histiocytic	34 (57%)	28 (47%)	34 (57%)
Inflammation, granulomatous			1 (2%)
Necrosis	2 (3%)		
Pigmentation	1 (2%)	34 (57%)	33 (55%)
Pigmentation, hemosiderin	9 (15%)	25 (42%)	36 (60%)
Capsule, hemorrhage	1 (2%)		
Capsule, necrosis	1 (2%)		
Thymus	(55)	(56)	(58)
Cyst	1 (2%)	1 (2%)	
Depletion lymphoid	1 (2%)	1 (2%)	
Pigmentation	5 (9%)	34 (61%)	41 (71%)
Epithelial cell, hyperplasia	1 (2%)		

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Integumentary System			
Mammary gland	(56)	(56)	(49)
Galactocele	21 (38%)	12 (21%)	7 (14%)
Acinus, hyperplasia			1 (2%)
Skin	(60)	(61)	(58)
Abscess	1 (2%)		
Musculoskeletal System			
Bone	(60)	(61)	(60)
Hyperplasia			1 (2%)
Necrosis			1 (2%)
Nervous System			
Brain	(60)	(61)	(60)
Hemorrhage		1 (2%)	
Necrosis		1 (2%)	
Respiratory System			
Lung	(60)	(61)	(60)
Atelectasis	3 (5%)	5 (8%)	5 (8%)
Bacterium	1 (2%)		
Congestion	3 (5%)	4 (7%)	3 (5%)
Edema	3 (5%)	5 (8%)	6 (10%)
Hemorrhage	2 (3%)	7 (11%)	3 (5%)
Infiltration cellular, histiocytic	22 (37%)	48 (79%)	46 (77%)
Inflammation, acute	2 (3%)	4 (7%)	9 (15%)
Inflammation, granulomatous		3 (5%)	8 (13%)
Metaplasia, osseous	1 (2%)		
Pigmentation	1 (2%)	23 (38%)	35 (58%)
Pigmentation, cholesterol			1 (2%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	4 (7%)
Nose	(60)	(61)	(60)
Fungus	1 (2%)	2 (3%)	1 (2%)
Hemorrhage	2 (3%)		
Inflammation, acute	7 (12%)	13 (21%)	15 (25%)
Submucosa, glands, hyperplasia			1 (2%)
Trachea	(60)	(59)	(60)
Erosion			1 (2%)
Inflammation, acute		1 (2%)	1 (2%)
Inflammation, chronic active			1 (2%)
Metaplasia, squamous			1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Titanocene Dichloride (continued)

	Vehicle Control	25 mg/kg	50 mg/kg
Special Senses System			
Ear		(1)	(6)
Inflammation, acute			1 (17%)
Canal, abscess		1 (100%)	2 (33%)
Eye	(5)	(11)	(18)
Hemorrhage			1 (6%)
Inflammation, acute		1 (9%)	1 (6%)
Synechia	2 (40%)	1 (9%)	3 (17%)
Lens, cataract	1 (20%)	5 (45%)	4 (22%)
Retina, atrophy	3 (60%)		2 (11%)
Harderian gland	(4)	(8)	(14)
Pigmentation		1 (13%)	5 (36%)
Urinary System			
Kidney	(60)	(61)	(60)
Bacterium	1 (2%)		
Cyst		1 (2%)	
Inflammation, chronic active	1 (2%)		
Nephropathy	32 (53%)	26 (43%)	35 (58%)
Cortex, mineralization	4 (7%)	4 (7%)	2 (3%)
Papilla, mineralization	5 (8%)	10 (16%)	6 (10%)
Pelvis, mineralization	2 (3%)		
Renal tubule, hyperplasia			1 (2%)
Renal tubule, pigmentation	35 (58%)	32 (52%)	40 (67%)
Urinary bladder	(60)	(58)	(60)
Hemorrhage	1 (2%)		

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Ames *et al.* (1975) with modifications as listed below and described in greater detail in Haworth *et al.* (1983). Titanocene dichloride was sent to the laboratory as a coded aliquot from Radian Corporation, Austin, TX. The test chemical was incubated with the *Salmonella typhimurium* tester strain (TA98, TA100, TA1535, TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C prior to the addition of soft agar supplemented with ℓ-histidine and *d*-biotin, and subsequent plating on minimal glucose agar plates. Incubation was continued for an additional 48 hours.

In this assay, each test consisted of triplicate plates of concurrent positive and negative controls and of at least 5 doses of the test chemical. High dose was limited by toxicity. All positive assays were repeated under the conditions that elicited the positive response.

A positive response was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment.

CHINESE HAMSTER OVARY CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1985, 1987) and presented briefly below. Titanocene dichloride was sent to the laboratory as a coded aliquot from Radian Corporation, Austin, TX. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges and chromosomal aberrations both in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least 3 doses of the test chemical; the high dose was limited by toxicity.

In the sister chromatid exchange (SCE) test without S9, CHO cells were incubated for 26 hours with the study chemical in McCoy's 5A medium supplemented with 10% fetal bovine serum, ℓ-glutamine (2 mM), and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing the test chemical was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 more hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with the test chemical, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no test chemical and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining procedures were the same as for cells treated without S9.

In the chromosomal aberration (Abs) test without S9, cells were incubated in McCoy's 5A medium with the test chemical for 16 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with the test chemical and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 11 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test. If cell cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. For the SCE test, usually 50 second-division metaphase cells were scored for frequency of SCE per cell from each dose level; 200 first-division metaphase cells were scored at each dose level for the Abs test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on the slopes of the dose-response curves and on the individual dose points. A sister chromatid exchange frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Chromosomal aberration data are presented as percentage of cells with aberrations. As with SCE, both the dose-response curve and individual dose points were statistically analyzed.

Results

Titanocene dichloride was tested at concentrations of up to 3,333 μg/plate for the induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 both in the presence and in the absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9, as shown in Table C1 (Haworth *et al.*, 1983). A positive response was observed only for the base-substitution strain TA100 tested in the absence of S9; all other strain/activation combinations yielded negative results. In cytogenetic tests with Chinese hamster ovary cells, titanocene dichloride was negative for the induction of sister chromatid exchanges and chromosomal aberrations, with and without metabolic activation (Tables C2 and C3). An increase in sister chromatid exchange frequency was observed at three of the four concentrations tested without S9, but these increases were not of sufficient magnitude to be considered a positive response and the results of the trend test were not significant. Doses in the sister chromatid exchange test ranged from 10 to 333 μg/mL without S9 and 33 to 1,000 μg/mL with S9. In the chromosomal aberration test without S9, doses ranged from 35 to 349 μg/mL and a delayed harvest protocol was used to allow sufficient metaphase cells to accumulate for analysis. In the presence of S9, doses of 162 to 750 μg/mL were tested; a precipitate formed at the two highest concentrations (349 and 750 μg/mL).

TABLE C1
Mutagenicity of Titanocene Dichloride in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9	+10% rat S9
TA100	0.0	91 ± 0.9	83 ± 7.6	104 ± 2.1	82 ± 5.1
	33.3	166 ± 9.7	102 ± 0.0	110 ± 9.2	93 ± 2.5
	100.0	178 ± 11.0	210 ± 8.5	91 ± 12.3	94 ± 4.6
	333.3	193 ± 9.3	271 ± 4.3	90 ± 8.4	89 ± 5.6
	1,000.0	184[c] ± 20.9	153[c] ± 13.8	101 ± 10.0	99 ± 7.2
	3,333.3	85[c] ± 11.6	64[c] ± 4.4	100 ± 13.4	93 ± 6.3
Trial Summary		Positive	Positive	Negative	Negative
Positive control[d]		352 ± 16.0	486 ± 21.5	1482 ± 54.3	338 ± 9.4
TA1535	0.0	11 ± 2.0		5 ± 0.3	7 ± 1.5
	33.3	18 ± 0.7		5 ± 0.9	7 ± 1.2
	100.0	13 ± 3.5		6 ± 2.0	7 ± 0.0
	333.3	13 ± 1.5		7 ± 0.7	12 ± 2.3
	1,000.0	14[c] ± 3.2		7 ± 0.6	15 ± 1.0
	3,333.3	4[c] ± 2.6		14 ± 5.0	16 ± 5.2
Trial summary		Negative		Negative	Negative
Positive control[d]		285 ± 7.8		404 ± 18.0	211 ± 12.4
TA1537	0.0	7 ± 0.7		7 ± 1.0	18 ± 0.3
	33.3	6 ± 0.7		5 ± 0.6	14 ± 2.3
	100.0	6 ± 1.8		6 ± 1.5	18 ± 1.2
	333.3	7 ± 1.2		4 ± 0.6	19 ± 1.0
	1,000.0	7[c] ± 1.5		5 ± 0.6	16 ± 2.0
	3,333.3	5[c] ± 0.7		4 ± 1.5	13 ± 1.9
Trial summary		Negative		Negative	Negative
Positive control[d]		312 ± 24.1		466 ± 35.1	83 ± 6.9
TA98	0.0	24 ± 4.2		33 ± 4.1	36 ± 3.1
	33.3	33 ± 4.6		27 ± 3.5	30 ± 1.2
	100.0	38 ± 1.5		25 ± 5.4	24 ± 2.0
	333.3	30 ± 1.2		32 ± 2.6	26 ± 1.2
	1,000.0	29[c] ± 5.6		29 ± 1.9	28 ± 5.4
	3,333.3	11[c] ± 3.2		26 ± 3.6	21 ± 4.1
Trial summary		Equivocal		Negative	Negative
Positive control[d]		529 ± 11.8		1,222 ± 59.4	153 ± 12.2

[a] Study performed at SRI International. These data and the detailed protocol are presented in Haworth *et al.* (1983). Cells and the test chemical or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity or solubility, but did not exceed 10 mg/plate; 0 μg/plate dose is the solvent control.

[b] Revertants are presented as mean ± the standard error from 3 plates.

[c] Slight toxicity

[d] 2-aminoanthracene was used for all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE C2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Titanocene Dichloride[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Increase over Solvent (%)[b]
-S9[c]								
Trial 1--Summary: Negative								
Dimethylsulfoxide		50	1,045	419	0.40	8.4	26.0	
Mitomycin-C	0.0010	50	1,051	696	0.66	13.9	26.0	65.16
	0.0050	10	209	280	1.33	28.0	26.0	234.13
Titanocene dichloride	10.0	50	1,046	479	0.45	9.6	26.0	14.21
	33.0	50	1,043	450	0.43	9.0	26.0	7.60
	100.0	50	1,047	479	0.45	9.6	26.0	14.10
	333.0	0						
								P=0.060[d]
+S9[e]								
Trial 1--Summary: Negative								
Dimethylsulfoxide		50	1,054	481	0.45	9.6	26.0	
Cyclophosphamide	0.1250	50	1,045	705	0.67	14.1	26.0	47.83
	0.5000	10	209	219	1.04	21.9	26.0	129.61
Titanocene dichloride	33.0	50	1,048	468	0.44	9.4	26.0	-2.15
	100.0	50	1,054	490	0.46	9.8	26.0	1.87
	333.0	50	1,046	432	0.41	8.6	26.0	-9.50
	1,000.0	0						
								P=0.891

[a] Study performed at Sitek Research Laboratories. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with the test chemical or solvent (dimethylsulfoxide) as described in [c] and [d] below, and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air dried, and stained.

[b] Percentage increase in SCEs/chromosome of culture exposed to the test chemical relative to those of culture exposed to solvent.

[c] In the absence of S9, cells were incubated with the test chemical or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 hours.

[d] Significance of relative SCEs/chromosome tested by linear regression trend test vs. log of the dose

[e] In the presence of S9, cells were incubated with the test chemical or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with Colcemid present for the final 2 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

TABLE C3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Titanocene Dichloride[a]

-S9[b]					+S9[c]				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs[d]
Trial 1--Summary: Negative					**Trial 1--Summary: Negative**				
Harvest time: 18.5 hours					Harvest time: 13.0 hours				
Dimethylsulfoxide	200	3	0.02	1.5	Dimethylsulfoxide	200	2	0.01	1.0
Mitomycin-C					Cyclophosphamide				
0.4000	50	37	0.74	60.0	20	25	21	0.84	48.0
Titanocene dichloride					Titanocene dichloride				
35	200	2	0.01	1.0	162	200	1	0.01	0.5
75	200	1	0.01	0.5	349[e]	200	1	0.01	0.5
162	200	2	0.01	1.0	750[e]	200	1	0.01	0.5
349	0								
				P=0.737					P=0.726

[a] Study performed at Sitek Research Laboratories. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with the test chemical or solvent (dimethylsulfoxide) as indicated in [b] and [c]. Cells were arrested in the first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[b] In the absence of S9, cells were incubated with the test chemical or solvent for 16 hours at 37° C. Because of significant chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to 16 hours to allow sufficient metaphases at harvest. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 to 3 hours followed by harvest.

[c] In the presence of S9, cells were incubated with the test chemical or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 11 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[d] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose

[e] Precipitate formed at these concentrations

APPENDIX D
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE D1a
Organ Weights for Rats in the 14-Day Gavage Studies of Titanocene Dichloride[a]

	Vehicle Control	62 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n	5	5	5	5	1	0
Necropsy body wt	220 ± 5	218 ± 8	198 ± 6*	178 ± 6**	100[b]	–[c]
Brain	1.83 ± 0.02	1.78 ± 0.02	1.71 ± 0.07	1.79 ± 0.03	1.71[b]	–
Heart	0.75 ± 0.01	0.77 ± 0.05	0.59 ± 0.08**	0.60 ± 0.03**	0.47[b]	–
Kidney	0.85 ± 0.03	0.79 ± 0.03	0.70 ± 0.07*	0.72 ± 0.04*	0.60[b]	–
Liver	9.73 ± 0.53	9.59 ± 0.36	9.13 ± 0.39	7.96 ± 0.35*	5.15[b]	–
Lung	1.21 ± 0.07	1.16 ± 0.07	1.00 ± 0.03*	0.92 ± 0.06**	0.93[b]	–
Testis	1.26 ± 0.03	1.28 ± 0.03	1.26 ± 0.02	1.20 ± 0.04	0.47[b]	–
Thymus	0.46 ± 0.03	0.39 ± 0.05	0.32 ± 0.05*	0.32 ± 0.03*	0.10[b]	–
Female						
n	5	5	5	5	3	0
Necropsy body wt	131 ± 4	131 ± 3	117 ± 5	110 ± 4**	87 ± 6**	–[c]
Brain	1.64 ± 0.03	1.63 ± 0.02	1.58 ± 0.02	1.58 ± 0.01	1.55 ± 0.02*	–
Heart	0.46 ± 0.01	0.44 ± 0.01	0.37 ± 0.02**	0.34 ± 0.01**	0.31 ± 0.01**	–
Kidney	0.48 ± 0.02	0.50 ± 0.01	0.44 ± 0.02	0.41 ± 0.00*	0.41 ± 0.01*	–
Liver	5.06 ± 0.26	5.52 ± 0.18	5.25 ± 0.20	4.75 ± 0.29	4.12 ± 0.26	–
Lung	0.74 ± 0.02	0.70 ± 0.01	0.67 ± 0.04	0.60 ± 0.03**	0.60 ± 0.02*	–
Thymus	0.31 ± 0.01	0.29 ± 0.02	0.24 ± 0.02**	0.19 ± 0.02**	0.14 ± 0.05**	–

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Organ weights are given in grams (mean ± standard error).
[b] No standard error calculated due to high mortality in this group
[c] No data calculated due to 100% mortality in this group

TABLE D1b
Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Gavage Studies of Titanocene Dichloride[a]

	Vehicle Control	62 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n	5	5	5	5	1	0
Necropsy body wt	220 ± 5	218 ± 8	198 ± 6*	178 ± 6**	100[b]	–[c]
Brain	8.34 ± 0.23	8.21 ± 0.24	8.68 ± 0.43	10.11 ± 0.37**	17.04[b]	–
Heart	3.43 ± 0.08	3.53 ± 0.16	2.99 ± 0.40	3.37 ± 0.20	4.70[b]	–
Kidney	3.87 ± 0.10	3.66 ± 0.05	3.51 ± 0.34	4.04 ± 0.24	5.99[b]	–
Liver	44.2 ± 1.8	44.1 ± 0.9	46.1 ± 1.5	44.7 ± 0.6	51.4[b]	–
Lung	5.50 ± 0.28	5.30 ± 0.21	5.07 ± 0.20	5.18 ± 0.27	9.32[b]	–
Testis	5.74 ± 0.04	5.91 ± 0.15	6.37 ± 0.08**	6.75 ± 0.25**	4.65[b]	–
Thymus	2.10 ± 0.13	1.77 ± 0.24	1.61 ± 0.25	1.84 ± 0.20	1.02[b]	–
Female						
n	5	5	5	5	3	0
Necropsy body wt	131 ± 4	131 ± 3	117 ± 5	110 ± 4**	87 ± 6**	–[c]
Brain	12.6 ± 0.2	12.4 ± 0.3	13.5 ± 0.5	14.4 ± 0.4**	18.0 ± 1.3**	–
Heart	3.52 ± 0.09	3.40 ± 0.10	3.19 ± 0.09*	3.05 ± 0.06**	3.63 ± 0.16	–
Kidney	3.70 ± 0.06	3.81 ± 0.10	3.74 ± 0.10	3.75 ± 0.11	4.75 ± 0.42**	–
Liver	38.7 ± 1.1	42.1 ± 0.5*	44.9 ± 2.0*	43.0 ± 1.8	47.5 ± 0.9**	–
Lung	5.68 ± 0.18	5.38 ± 0.09	5.75 ± 0.22	5.40 ± 0.20	6.95 ± 0.63	–
Thymus	2.38 ± 0.07	2.18 ± 0.12	2.06 ± 0.14	1.72 ± 0.16**	1.59 ± 0.57	–

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] No standard error calculated due to high mortality in this group
[c] No data calculated due to 100% mortality in this group

TABLE D2a
Organ Weights for Rats in the 13-Week Gavage Studies of Titanocene Dichloride[a]

	Vehicle Control	8 mg/kg	16 mg/kg	31 mg/kg	62 mg/kg	125 mg/kg
Male						
n	9	10	10	10	10	10
Necropsy body wt	378 ± 7	368 ± 5	368 ± 7[b]	368 ± 5	336 ± 9**	296 ± 6**
Brain	1.96 ± 0.03	1.97 ± 0.03	1.97 ± 0.02[b]	1.99 ± 0.01[b]	1.95 ± 0.02	1.93 ± 0.03
Heart	1.13 ± 0.04	1.00 ± 0.02*	1.05 ± 0.02*	1.07 ± 0.03	1.00 ± 0.03*[b]	0.85 ± 0.02**
Kidney	1.18 ± 0.04	1.34 ± 0.03*	1.30 ± 0.03	1.33 ± 0.03	1.28 ± 0.04	1.14 ± 0.03
Liver	15.44 ± 0.49[c]	15.42 ± 0.45	15.18 ± 0.51[b]	15.17 ± 0.56	13.45 ± 0.60	10.14 ± 0.22**
Lung	1.74 ± 0.09	1.92 ± 0.09	1.86 ± 0.07[b]	1.89 ± 0.06	1.74 ± 0.06	1.63 ± 0.05
Testis	1.50 ± 0.03	1.52 ± 0.02	1.53 ± 0.04	1.57 ± 0.01	1.54 ± 0.04	1.51 ± 0.03[b]
Thymus	308.6 ± 22.9	285.4 ± 16.2	301.8 ± 13.9	297.8 ± 11.8	242.0 ± 10.6**	200.0 ± 7.3**
Female						
n	10	10	10	10	10	9
Necropsy body wt	215 ± 3	213 ± 3	212 ± 2	207 ± 3	199 ± 3**	193 ± 2**
Brain	1.84 ± 0.02	1.82 ± 0.03	1.82 ± 0.02	1.79 ± 0.03	1.85 ± 0.02	1.77 ± 0.02
Heart	0.66 ± 0.02	0.66 ± 0.02	0.70 ± 0.02	0.66 ± 0.02	0.64 ± 0.01	0.60 ± 0.02
Kidney	0.72 ± 0.02	0.78 ± 0.01	0.77 ± 0.01	0.77 ± 0.02	0.74 ± 0.01	0.72 ± 0.02
Liver	7.48 ± 0.32	8.04 ± 0.13	8.56 ± 0.19**	7.51 ± 0.29	7.86 ± 0.19	7.58 ± 0.15
Lung	1.19 ± 0.04	1.17 ± 0.05	1.22 ± 0.04	1.19 ± 0.04	1.15 ± 0.04	1.13 ± 0.05
Thymus	266.9 ± 14.2	278.6 ± 17.9[b]	258.4 ± 10.6	234.2 ± 8.3	227.5 ± 8.3*	214.9 ± 10.7**

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Organ weights are given in grams (mean ± standard error), except thymus weights, which are given in milligrams.
[b] n=9
[c] n=8

TABLE D2b
Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Studies of Titanocene Dichloride[a]

	Vehicle Control	8 mg/kg	16 mg/kg	31 mg/kg	62 mg/kg	125 mg/kg
Male						
n	9	10	10	10	10	10
Necropsy body wt	378 ± 7	368 ± 5	368 ± 7	368 ± 5[b]	336 ± 9**	296 ± 6**
Brain	5.20 ± 0.08	5.36 ± 0.08	5.42 ± 0.09[b]	5.45 ± 0.06*[b]	5.83 ± 0.17**	6.53 ± 0.16**
Heart	3.00 ± 0.07	2.73 ± 0.05*	2.85 ± 0.07	2.90 ± 0.07	2.99 ± 0.06[b]	2.87 ± 0.03
Kidney	3.12 ± 0.12	3.65 ± 0.08**	3.54 ± 0.05**	3.62 ± 0.10**	3.82 ± 0.06**	3.85 ± 0.09**
Liver	40.9 ± 1.6[c]	41.9 ± 1.0	41.1 ± 1.6[b]	41.2 ± 1.6	40.0 ± 1.5	34.2 ± 0.5**
Lung	4.61 ± 0.25	5.20 ± 0.23	5.09 ± 0.16[b]	5.13 ± 0.15	5.20 ± 0.21	5.53 ± 0.24*
Testis	3.97 ± 0.10	4.15 ± 0.08	4.17 ± 0.08*	4.27 ± 0.07*	4.59 ± 0.06**	5.12 ± 0.11**[b]
Thymus	0.82 ± 0.06	0.78 ± 0.05	0.82 ± 0.04	0.81 ± 0.03	0.72 ± 0.03	0.68 ± 0.02**
Female						
n	10	10	10	10	10	9
Necropsy body wt	215 ± 3	213 ± 3	212 ± 2	207 ± 3	199 ± 3**	193 ± 2**
Brain	8.56 ± 0.17	8.52 ± 0.12	8.59 ± 0.11	8.68 ± 0.19	9.33 ± 0.19*	9.21 ± 0.08**
Heart	3.06 ± 0.08	3.12 ± 0.10	3.29 ± 0.09	3.19 ± 0.10	3.21 ± 0.05	3.09 ± 0.07
Kidney	3.34 ± 0.11	3.63 ± 0.03	3.65 ± 0.06	3.71 ± 0.08*	3.73 ± 0.05**	3.76 ± 0.08**
Liver	34.7 ± 1.4	37.7 ± 0.7*	40.4 ± 0.7**	36.4 ± 1.6*	39.6 ± 0.7**	39.3 ± 0.6**
Lung	5.54 ± 0.19	5.51 ± 0.26	5.74 ± 0.17	5.76 ± 0.21	5.79 ± 0.23	5.87 ± 0.25
Thymus	1.24 ± 0.06	1.30 ± 0.08	1.22 ± 0.05	1.13 ± 0.04	1.15 ± 0.04	1.11 ± 0.05

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9
[c] n=8

TABLE D3a
Organ Weights for Rats at the 15-Month Interim Evaluations in the 2-Year Gavage Studies of Titanocene Dichloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg
Male			
n	10	10	10
Necropsy body wt	509 ± 8	470 ± 7**	443 ± 13**
Brain	2.01 ± 0.03	2.01 ± 0.02[b]	1.98 ± 0.02
Heart	1.13 ± 0.07	1.05 ± 0.03	1.06 ± 0.07
Kidney	1.35 ± 0.03	1.27 ± 0.02*	1.13 ± 0.03**
Liver	15.21 ± 0.35	13.87 ± 0.25**	11.76 ± 0.34**
Lung	1.69 ± 0.21	1.45 ± 0.03	1.42 ± 0.06
Spleen	0.82 ± 0.02	0.78 ± 0.04[b]	0.68 ± 0.01**
Female			
n	10	10	10
Necropsy body wt	311 ± 6	298 ± 8	284 ± 10*
Brain	1.78 ± 0.03	1.83 ± 0.02[b]	1.81 ± 0.02
Heart	0.70 ± 0.01	0.69 ± 0.01[b]	0.71 ± 0.02
Kidney	0.81 ± 0.04	0.72 ± 0.02	0.73 ± 0.02
Liver	8.60 ± 0.22	8.75 ± 0.22	9.43 ± 0.19**
Lung	1.04 ± 0.09	0.96 ± 0.01	1.00 ± 0.02
Spleen	0.53 ± 0.02	0.53 ± 0.02	0.50 ± 0.01

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Organ weights and body weights are given in grams (mean ± standard error).
[b] n=9

TABLE D3b
Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluations in the 2-Year Gavage Studies of Titanocene Dichloride[a]

	Vehicle Control	25 mg/kg	50 mg/kg
Male			
n	10	10	10
Necropsy body wt	509 ± 8	470 ± 7**	443 ± 13**
Brain	3.97 ± 0.07	4.29 ± 0.07**[b]	4.49 ± 0.11**
Heart	2.22 ± 0.13	2.24 ± 0.05	2.39 ± 0.15
Kidney	2.65 ± 0.06	2.70 ± 0.04	2.56 ± 0.05
Liver	29.9 ± 0.4	29.5 ± 0.5	26.6 ± 0.5**
Lung	3.32 ± 0.41	3.09 ± 0.06	3.21 ± 0.15
Spleen	1.61 ± 0.04	1.66 ± 0.07[b]	1.53 ± 0.03
Female			
n	10	10	10
Necropsy body wt	311 ± 6	298 ± 8	284 ± 10*
Brain	5.73 ± 0.13	6.17 ± 0.17[b]	6.43 ± 0.21**
Heart	2.26 ± 0.06	2.34 ± 0.04[b]	2.53 ± 0.08**
Kidney	2.60 ± 0.12	2.41 ± 0.03	2.62 ± 0.10
Liver	27.7 ± 0.4	29.4 ± 0.4*	33.6 ± 1.2**
Lung	3.37 ± 0.33	3.26 ± 0.07	3.56 ± 0.12**
Spleen	1.72 ± 0.06	1.79 ± 0.08	1.77 ± 0.07

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

APPENDIX E
ANALYSIS OF TISSUE RESIDUES FOR TITANIUM IN THE GAVAGE STUDIES

ANALYSIS OF TISSUE RESIDUES FOR TITANIUM IN THE GAVAGE STUDIES

Preparation and Analysis of Tissue Residues for Titanium

Tissue samples from the heart, lung, liver, and spleen were obtained from the study laboratory. The tissues were frozen in liquid nitrogen immediately after removal from the animals and stored at approximately -70° C until shipment to the analytical chemistry laboratory, Midwest Research Institute (MRI), Kansas City, MO. Reports of analyses performed in support of the studies are on file at NIEHS.

In the analysis of the 14-day and 13-week study samples, the weighed tissues were digested in 2 mL of a 2:1 mixture of ULTREX sulfuric and nitric acids, and then 1 mL of water was added. Digestion was allowed to proceed for 5 hours in a shaker bath at 55° C, then the samples were analyzed using a Jarrell-Ash Atomcomp Linear 1100 inductively coupled plasma-emission spectrometer set at 334.9 nm. Quantitation was achieved from the standard curve analyzed using linear regression analysis of digested solution standards. The method was validated by analyzing spiked control tissues. The precision ranged from 4.2% to 11.6% RSD and the average recovery was 94%.

Tissue samples from the 15-month and 2-year studies were weighed and then digested in 4 to 5 mL of a solution of approximately 2.5 μg/mL yttrium (as the internal standard) in 50% nitric acid, and allowing samples to equilibrate at room temperature for 30 minutes. Samples were then heated at 110° C for one and a half hours, until a clear solution was obtained. Sample weights were then adjusted to 10 grams with water and the samples were reheated for 15 minutes at 110° C. Titanium concentrations of the digested tissue samples were determined with a Jarrell-Ash Model 1155A inductively coupled plasma-emission spectrometer, and the emissions were monitored at 334.9 nm for titanium and 371.0 nm for yttrium. Five titanium solution standards along with four spiked NBS reference samples were analyzed concomitantly with the tissue samples. Sample tissue concentrations of titanium were determined from the standard curve developed using linear regression analysis. The method was validated by analyzing solution standards and NBS standard reference materials. The precision ranged from 1% to 20% and the average recovery was 89%.

Results

The results for the tissue analyses for titanium of the 14-day, 13-week, and 2-year studies are presented in Tables E1, E2, E3, and E4.

TABLE E1
Tissue Residues of Titanium in Rats in the 14-Day Gavage Studies of Titanocene Dichloride[a]

Dose	Vehicle Control[b]	250 mg/kg	500 mg/kg
Male			
Heart	<DL[c]	1.74 ± 0.08	4.20[d]
Liver	<DL[e]	7.98 ± 0.53	28.60[d]
Lung	<DL	3.38 ± 0.15	7.70[d]
Spleen	<DL	9.64 ± 0.46	41.30[d]
Female			
Heart	<DL	–[f]	3.47 ± 0.30[e]
Liver	<DL[g]	–	18.50 ± 1.61[e]
Lung	<DL	–	5.83 ± 0.32[e]
Spleen	<DL	–	22.90 ± 1.40[g]

[a] Mean ± standard error for groups of 5 animals unless otherwise specified. Tissue residues are expressed as ppm (μg titanium per g of tissue). Tissue samples were analyzed for titanium by inductively coupled plasma-atomic emission spectroscopy following a wet digestion procedure.
[b] In one male rat, concentrations of approximately 1 ppm were observed in all four tissues.
[c] Detection limit (DL) estimated at 0.03 ppm.
[d] n=1
[e] n=3
[f] No measurements taken for this sex at this dose level.
[g] n=2

TABLE E2
Tissue Residues of Titanium in Rats in the 13-Week Gavage Studies of Titanocene Dichloride[a]

Dose	Vehicle Control	31 mg/kg	125 mg/kg
Male			
Heart	<DL[b]	2.22 ± 0.08	7.26 ± 0.25
Liver	0.12 ± 0.07	5.60 ± 0.24	25.00 ± 1.19
Lung	<DL	3.82 ± 0.20	12.70 ± 0.76
Spleen	<DL	17.30 ± 0.92	63.80 ± 3.96
Female			
Heart	<DL[c]	2.24 ± 0.05	5.92 ± 0.20
Liver	0.08 ± 0.02	6.88 ± 0.35	21.08 ± 1.64[c]
Lung	<DL	3.76 ± 0.16	15.16 ± 4.46
Spleen	<DL	18.68 ± 0.96	52.20 ± 1.86

[a] Mean ± standard error for groups of 5 animals unless otherwise specified. Tissue residues are expressed as ppm (μg titanium per g of tissue).
[b] Detection limit (DL) estimated at 0.04 ppm.
[c] n=4

TABLE E3
Tissue Residues of Titanium in Rats at the 15-Month Interim Evaluations in the 2-Year Gavage Studies of Titanocene Dichloride[a]

Dose	Vehicle Control	25 mg/kg	50 mg/kg
Male			
Heart	<DL[b]	14.61 ± 0.36[c]	22.34 ± 0.74
Liver	<DL	21.40 ± 0.57	32.88 ± 1.03
Lung	<DL	22.92 ± 1.89	38.76 ± 5.51
Spleen	<DL[c]	99.22 ± 2.60[c]	178.72 ± 5.44
Female			
Heart	<DL	14.51 ± 0.37	23.71 ± 0.65
Liver	<DL	23.36 ± 0.45	25.72 ± 0.86
Lung	<DL	28.77 ± 7.58	41.81 ± 3.41
Spleen	<DL	125.00 ± 3.96	227.34 ± 4.49

[a] Mean ± standard error for groups of 10 animals unless otherwise specified. Tissue residues are expressed as ppm (μg titanium per g of tissue).
[b] Detection limit (DL) estimated at 0.3 ppm.
[c] n=9

TABLE E4
Tissue Residues of Titanium in Rats in the 2-Year Gavage Studies of Titanocene Dichloride[a]

Dose	Vehicle Control	25 mg/kg	50 mg/kg
Male			
Heart	<DL[b]	23.10 ± 0.81	32.20 ± 1.78
Liver	<DL	22.30 ± 0.70	31.40 ± 1.38
Lung	<DL	26.30 ± 3.79	29.80 ± 3.32
Spleen	<DL	108.50 ± 9.99	165.22 ± 32.17[c]
Female			
Heart	<DL	19.70 ± 1.22	29.40 ± 1.23
Liver	<DL	25.70 ± 0.76	35.60 ± 4.01
Lung	<DL	20.70 ± 1.65	31.70 ± 2.97
Spleen	<DL	148.30 ± 23.48	257.30 ± 31.45

[a] Mean ± standard error for groups of 10 animals unless otherwise specified. Tissue residues are expressed as ppm (μg titanium per g of tissue).
[b] Detection limit (DL) estimated at 3 ppm.
[c] n=9

APPENDIX F
HEMATOLOGY, URINALYSIS, AND CLINICAL CHEMISTRY RESULTS

TABLE F1
Hematology Data for Rats in the 14-Day Gavage Studies of Titanocene Dichloride[a]

Analysis	Vehicle Control	62 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Male					
n	4	5	5	3	1
Hematocrit (%)	46.9 ± 1.9	41.7 ± 0.5*	39.4 ± 0.8**	38.9 ± 0.7**	35.8[c]
Hematocrit (manual) (%)	45.3 ± 0.3	42.4 ± 0.6*	43.0 ± 0.5*	43.0 ± 0.5*[d]	40.0[b]
Hemoglobin (g/dL)	15.9 ± 0.2	15.7 ± 0.2	15.1 ± 0.1**	15.1 ± 0.2*	13.7[b]
Erythrocytes ($10^6/\mu L$)	8.67 ± 0.17	8.73 ± 0.14	8.38 ± 0.20	8.35 ± 0.12	7.41[b]
Leukocytes ($10^3/\mu L$)	6.00 ± 0.23	5.80 ± 0.39	5.54 ± 0.45	5.63 ± 0.68	5.70[b]
Segmented neutrophils ($10^3/\mu L$)	1.13 ± 0.07	1.22 ± 0.25	1.31 ± 0.29	1.20 ± 0.16	1.03[b]
Lymphocytes ($10^3/\mu L$)	4.74 ± 0.15	4.40 ± 0.17	4.11 ± 0.40	4.33 ± 0.60	4.56[b]
Monocytes ($10^3/\mu L$)	0.12 ± 0.06	0.14 ± 0.06	0.12 ± 0.06	0.10 ± 0.05	0.11[b]
Eosinophils ($10^3/\mu L$)	0.02 ± 0.02	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00[b]
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.60 ± 0.60	0.80 ± 0.49	1.00 ± 0.58	0.00[b]
Female					
n	5	5	5	5	2
Hematocrit (%)	48.0 ± 1.0	46.9 ± 1.4	44.3 ± 1.6	42.8 ± 1.3**	38.7 ± 1.8**
Hematocrit (manual) (%)	45.6 ± 1.0	46.2 ± 0.8	45.2 ± 0.9	43.4 ± 1.3	39.0 ± 1.0*
Hemoglobin (g/dL)	17.8 ± 0.2	17.6 ± 0.2	17.3 ± 0.3	16.3 ± 0.3**	15.4 ± 0.3**
Erythrocytes ($10^6/\mu L$)	9.40 ± 0.05	9.47 ± 0.26	9.06 ± 0.18	9.17 ± 0.30	8.16 ± 0.38*
Leukocytes ($10^3/\mu L$)	5.58 ± 0.23	7.56 ± 0.41*	7.28 ± 0.59	5.30 ± 0.39	7.25 ± 0.65
Segmented neutrophils ($10^3/\mu L$)	0.90 ± 0.11	1.80 ± 0.22*	1.46 ± 0.25	0.99 ± 0.09	1.75 ± 0.10
Lymphocytes ($10^3/\mu L$)	4.61 ± 0.19	5.60 ± 0.25	5.69 ± 0.65	4.21 ± 0.37	5.29 ± 0.80
Monocytes ($10^3/\mu L$)	0.07 ± 0.02	0.10 ± 0.02	0.14 ± 0.05	0.10 ± 0.01	0.21 ± 0.05*
Nucleated erythrocytes/ 100 leukocytes	0.60 ± 0.40	0.20 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. No data calculated in 1,000 mg/kg group due to 100% mortality in this group.
[b] No standard error calculated due to high mortality in this group
[c] n=5

TABLE F2
Clinical Chemistry Data for Rats in the 14-Day Gavage Studies of Titanocene Dichloride[a]

Analysis	Vehicle Control	62 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Male					
n	5	5	5	5	1[b]
Blood urea nitrogen (mg/dL)	21.6 ± 1.2	21.2 ± 0.8	18.6 ± 1.0	19.8 ± 0.6	35.0
Creatinine (mg/dL)	0.22 ± 0.02	0.22 ± 0.02	0.20 ± 0.00	0.22 ± 0.02	0.10
Sodium (meq/L)	147 ± 0	148 ± 0	147 ± 0	145 ± 0*	150
Potassium (meq/L)	3.70 ± 0.14	3.60 ± 0.15	3.56 ± 0.16	3.54 ± 0.20	3.60
Chloride (meq/L)	106 ± 0	107 ± 1	109 ± 1*	110 ± 1**	115
Carbon dioxide (meq/L)	17.36 ± 0.92	19.54 ± 1.04	25.20 ± 1.00**	18.10 ± 1.12	19.90
Calcium (mg/dL)	10.36 ± 0.09	9.86 ± 0.04**	9.90 ± 0.14*	9.30 ± 0.11**	8.20
Phosphorus (mg/dL)	8.50 ± 0.10	8.12 ± 0.20	8.22 ± 0.42	7.70 ± 0.31	7.20
Total protein (g/dL)	6.32 ± 0.11	5.66 ± 0.04**	5.14 ± 0.14**	4.84 ± 0.07**	4.20
Albumin (g/dL)	4.48 ± 0.07	4.20 ± 0.08*	4.00 ± 0.16*	4.06 ± 0.05**	3.20
Globulin (g/dL)	1.84 ± 0.12	1.46 ± 0.05*	1.14 ± 0.04**	0.78 ± 0.10**	1.00
A/G Ratio	2.48 ± 0.16	2.90 ± 0.16	3.54 ± 0.25**	5.62 ± 0.85**	3.20
Total bilirubin (mg/dL)	0.5 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.3
Direct bilirubin (mg/dL)	0.02 ± 0.01	0.04 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.08
Cholesterol (U/mL)	0.46 ± 0.01	0.43 ± 0.02	0.43 ± 0.03	0.39 ± 0.01**	0.24
ALT (IU/L)	34 ± 2	53 ± 2**	47 ± 4*	45 ± 2*	58
AST (IU/L)	85 ± 4	107 ± 4	99 ± 9	80 ± 4	63
LDH (IU/L)	628 ± 41	1,106 ± 97	746 ± 79	440 ± 43	250
OCT (IU/L)	2.47 ± 0.67	2.01 ± 0.29	1.80 ± 0.27	1.38 ± 0.34	1.09
SDH (IU/L)	341 ± 19	445 ± 42	311 ± 52	175 ± 34*	87
pH	7.44 ± 0.02[c]	7.48 ± 0.01	7.47 ± 0.01	7.45 ± 0.01[c]	7.41
Female					
n	4	5	5	5	3
Blood urea nitrogen (mg/dL)	10.3 ± 0.3	11.8 ± 0.6	11.2 ± 0.7	10.6 ± 0.8	19.3 ± 6.3*
Creatinine (mg/dL)	0.40 ± 0.00	0.46 ± 0.05	0.42 ± 0.02	0.30 ± 0.00*	0.40 ± 0.00
Sodium (meq/L)	153 ± 1	154 ± 1	151 ± 0	152 ± 1	151 ± 1
Potassium (meq/L)	3.48 ± 0.11	3.78 ± 0.10	3.46 ± 0.14	3.30 ± 0.15	4.60 ± 0.10
Chloride (meq/L)	117 ± 1	121 ± 2	116 ± 1	118 ± 1	120 ± 3
Carbon dioxide (meq/L)	15.00 ± 2.43	15.14 ± 2.90	16.78 ± 1.71	21.94 ± 0.36*	19.07 ± 1.52
Calcium (mg/dL)	9.70 ± 0.28	10.76 ± 0.20	10.38 ± 0.19	10.40 ± 0.38	9.73 ± 0.32
Phosphorus mg/dL)	6.83 ± 0.13	7.24 ± 0.31	7.92 ± 0.40	6.52 ± 0.72	6.90 ± 0.83
Total protein (g/dL)	5.28 ± 0.05	5.08 ± 0.12	4.88 ± 0.13*	4.70 ± 0.15**	4.10 ± 0.15**
Albumin (g/dL)	3.55 ± 0.18	3.24 ± 0.11	3.46 ± 0.14	3.64 ± 0.13	3.27 ± 0.19
Globulin (g/dL)	1.73 ± 0.22	1.82 ± 0.09	1.42 ± 0.07	1.06 ± 0.04**	0.83 ± 0.12**
A/G Ratio	2.25 ± 0.50	1.80 ± 0.13	2.47 ± 0.18	3.44 ± 0.11*	4.15 ± 0.81*
Total bilirubin (mg/dL)	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.6 ± 0.1**
Direct bilirubin (mg/dL)	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.06 ± 0.01*	0.13 ± 0.02**
Cholesterol (U/mL)	1.43 ± 0.14	1.00 ± 0.10*	0.64 ± 0.08**	0.52 ± 0.06**	0.38 ± 0.06**
ALT (IU/L)	34 ± 2	37 ± 1	49 ± 4**	50 ± 3**	57 ± 5**
AST (IU/L)	102 ± 5	94 ± 4	86 ± 5	88 ± 3	102 ± 16
LDH (IU/L)	940 ± 107	782 ± 34	252 ± 15**	388 ± 35**	670 ± 23*
OCT (IU/L)	1.43 ± 0.52	1.14 ± 0.30	1.21 ± 0.16	2.25 ± 0.62	1.38 ± 0.17
SDH (IU/L)	394 ± 36	281 ± 44	446 ± 61	336 ± 49	275 ± 59
pH	7.48 ± 0.01	7.49 ± 0.01[c]	7.50 ± 0.01[c]	7.46 ± 0.01	7.54[d]

TABLE F2
Clinical Chemistry Data for Rats in the 14-Day Gavage Studies of Titanocene Dichloride[a]
(continued)

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error. No data calculated in 1,000 mg/kg group due to 100% mortality. A/G = albumin/globulin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; OCT = ornithine carbamoyltransferase; SDH = sorbitol dehydrogenase.
[b] No standard error calculated due to high mortality in this group
[c] n=4
[d] n=1

TABLE F3
Urinalysis Data for Rats in the 14-Day Gavage Studies of Titanocene Dichloride[a]

Analysis	Vehicle Control	62 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Male					
n	5	5	5	5	2
Specific gravity	1.046 ± 0.005	1.035 ± 0.006	1.026 ± 0.004*	1.041 ± 0.012	1.026 ± 0.002
Urine pH	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Female					
n	5	5	5	5	3
Specific gravity	1.025 ± 0.005	1.033 ± 0.007	1.022 ± 0.004	1.026 ± 0.006	1.012 ± 0.007
Urine pH	6.20 ± 0.20	6.40 ± 0.24	6.20 ± 0.20	6.20 ± 0.20	7.00 ± 0.00

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
[a] Mean ± standard error. No data calculated in 1,000 mg/kg group due to 100% mortality.

TABLE F4
Hematology Data for Rats at the 15-Month Interim Evaluations in the 2-Year Gavage Studies of Titanocene Dichloride[a]

Analysis	Vehicle Control	25 mg/kg	50 mg/kg
Male			
n	10	10	10
Hematocrit (%)	47.7 ± 1.1	44.0 ± 0.9*	42.3 ± 0.6**
Hemoglobin (g/dL)	15.9 ± 0.2	15.2 ± 0.3	14.5 ± 0.2**
Erythrocytes ($10^6/\mu L$)	9.62 ± 0.17	9.75 ± 0.17	9.75 ± 0.20
MCV (μ^3)	49.5 ± 0.9	45.2 ± 0.8**	43.5 ± 0.9**
MCH (pg)	16.5 ± 0.2	15.6 ± 0.3**	14.9 ± 0.4**
MCHC (g/dL)	33.3 ± 0.6	34.5 ± 0.5	34.3 ± 0.4
Platelets ($10^3/\mu L$)	520 ± 41	495 ± 19	406 ± 13**
Reticulocytes ($10^6/\mu L$)	0.21 ± 0.01	0.25 ± 0.02	0.26 ± 0.02
Leukocytes ($10^3/\mu L$)	3.95 ± 0.29	3.12 ± 0.20*	2.85 ± 0.18**
Segmented neutrophils ($10^3/\mu L$)	1.35 ± 0.16	0.87 ± 0.11*	0.80 ± 0.07**
Bands ($10^3/\mu L$)	0.09 ± 0.02	0.07 ± 0.02	0.05 ± 0.02
Lymphocytes ($10^3/\mu L$)	2.45 ± 0.17	2.06 ± 0.19	1.89 ± 0.16*
Monocytes ($10^3/\mu L$)	0.08 ± 0.03	0.09 ± 0.03	0.08 ± 0.02
Eosinophils $10^3/\mu L$)	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Female			
n	10	10	10
Hematocrit (%)	43.6 ± 0.4	42.0 ± 0.4*	39.8 ± 0.4**
Hemoglobin (g/dL)	15.1 ± 0.1	15.0 ± 0.2	13.8 ± 0.1**
Erythrocytes ($10^6/\mu L$)	8.03 ± 0.07	8.20 ± 0.11	8.56 ± 0.09**
MCV (μ^3)	54.1 ± 0.3	51.2 ± 0.4**	46.5 ± 0.2**
MCH (pg)	18.8 ± 0.2	18.2 ± 0.2*	16.2 ± 0.2**
MCHC (g/dL)	34.7 ± 0.3	35.7 ± 0.3*	34.8 ± 0.3
Platelets ($10^3/\mu L$)	373 ± 34	372 ± 15	387 ± 12
Reticulocytes ($10^6/\mu L$)	0.21 ± 0.02	0.17 ± 0.01	0.26 ± 0.02
Leukocytes ($10^3/\mu L$)	1.75 ± 0.08	1.94 ± 0.09	1.84 ± 0.08
Segmented neutrophils ($10^3/\mu L$)	0.42 ± 0.02	0.59 ± 0.04**	0.60 ± 0.07**
Bands ($10^3/\mu L$)	0.05 ± 0.01	0.07 ± 0.01	0.09 ± 0.01**
Lymphocytes ($10^3/\mu L$)	1.22 ± 0.07	1.21 ± 0.09	1.08 ± 0.05
Monocytes ($10^3/\mu L$)	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
Eosinophils ($10^3/\mu L$)	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration.

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Titanocene Dichloride

Titanocene dichloride was obtained from Pfaltz and Bauer, Inc. (Waterbury, CT) and Strem Chemicals (Newbury Port, MA) in two lots. One lot (Pfaltz and Bauer, lot no. PB013180) was used for the 14-day and 13-week studies and the other (Strem Chemicals, lot no. 13574-S) was used for the 2-year studies. Identity, purity, and stability analyses were conducted at the analytical chemistry lab, Midwest Research Institute (MRI, Kansas City, MO). Reports of analyses performed in support of the studies are on file at NIEHS.

The study chemical, a dark red, microcrystalline solid, was identified as titanocene dichloride by infrared and nuclear magnetic resonance spectroscopy. The spectra were consistent with those expected for the structure of titanocene dichloride and with the literature (*Sadtler Standard Spectra*) (Figures G1 and G2).

The purity of lot no. PB013180 was determined by elemental analysis for carbon, hydrogen, titanium, and chlorine; the results were generally in agreement with theoretical values, but with an excess of titanium. Potentiometric titration with 0.1 N NaOH using a combination electrode indicated a purity of 98.4%.

The purity of lot no. 13574-S was determined to be greater than 99% based on titration, elemental analysis, and Karl Fischer water analysis. Potentiometric titration with 0.1 N NaOH indicated a purity of 100.7%. Results of elemental analyses of this lot were in agreement with theoretical values, and Karl Fischer water analysis indicated the presence of 0.096% water.

Stability studies performed using potentiometric titration with 0.1 N NaOH indicated that titanocene dichloride was stable as a bulk chemical for at least 2 weeks at temperatures between −20° C and 60° C when protected from light. Based on the stability study results, the bulk chemical was stored at 0° ± 5° C at the testing laboratory throughout the study period. The stability of the bulk chemical was monitored by elemental analysis and by titration periodically during all phases of the studies. No change in the study material was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing appropriate amounts of titanocene dichloride and Mazola® corn oil and briefly homogenizing the suspensions with a Polytron blender (Table G1). Dose formulations were stirred continuously during sampling to maintain homogeneity. Studies of the stability and homogeneity of dose formulations were performed at MRI. Samples were diluted in chloroform, filtered through a 0.5 μ Millipore filter into 5 mL septum vials, and analyzed by high performance liquid chromatography using a Varian 5000 liquid chromatograph with an ultraviolet detector (254 nm) and a mobile phase of 20% hexane and 80% chloroform:tetrahydrofuran (80:20). No decrease in titanocene dichloride concentration was found after storage of the solutions for 2 weeks in the dark at 5° C or 25° C, or under simulated animal dosing conditions (open to air and light for 3 hours). During the studies, the dose formulations were stored at 0° ± 5° C, brought to room temperature, and hand agitated prior to administration. Unused formulations were discarded 14 days from the date of preparation.

The study laboratory conducted periodic analyses of the titanocene dichloride dose formulations using high performance liquid chromatography or ultraviolet spectroscopy. During the 14-day studies, samples were diluted with chloroform and the absorbances measured at 256 nm in a Perkin-Elmer Lambda 3 spectrometer. During the 13-week studies, samples were also diluted in chloroform, but the three lowest concentrations (0.8 to 3.1 mg/mL) were analyzed by high-performance liquid chromatography and the three highest concentrations (6.2 to 25.0 mg/mL) were analyzed by ultraviolet spectroscopy. At the end of the subchronic studies, ultraviolet spectroscopy was used to analyze all dose formulations, but the diluent used was acetonitrile instead of chloroform and absorbance was measured at 252 nm instead of 256 nm. The results of analyses of dose formulations for the 14-day and 13-week studies are shown in Tables G2 and G3. During the 2-year studies, the dose formulations were analyzed by ultraviolet spectroscopy at approximately 8-week intervals. Samples were taken from the animal rooms at approximately 24-week intervals. The diluent and the wavelength used varied during the studies: chloroform was used as diluent for the first two analyses, after which acetonitrile was used; absorbances were measured at 256 nm until 16 months into the studies (28 June 1984), after which absorbance was measured at 246 nm. The dose formulations were within ± 10% of the target concentrations 96% (27/28) of the time during the 2-year studies (Table G4).

Each lot of corn oil vehicle used in these studies was analyzed for peroxides at monthly intervals by Official Method Cd 8-53 of the American Oil Chemists' Society (Mehlenbacher *et al.*, 1972). The peroxide content of the vehicle ranged from 1.00 to 3.65 meq/kg, well below the tolerance limit of 10 meq/kg. Results of periodic referee analyses of the dose formulations performed by MRI using an ultraviolet spectroscopy (Cary 219 spectrometer, acetonitrile as diluent, absorbance measured at 251 to 253 nm) were in agreement with the results from the study laboratory (Table G5).

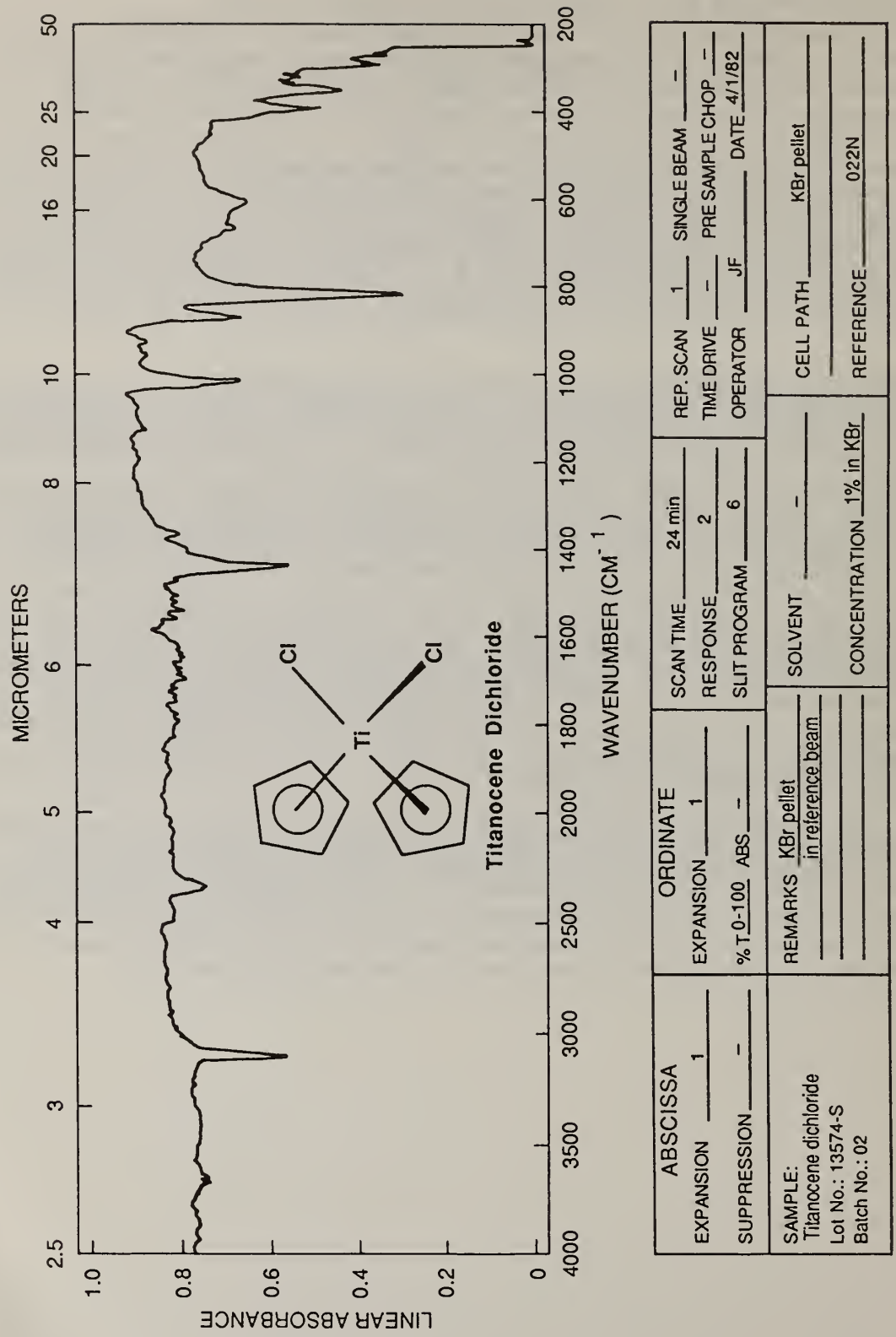

Figure G1
Infrared Spectrum of Titanocene Dichloride

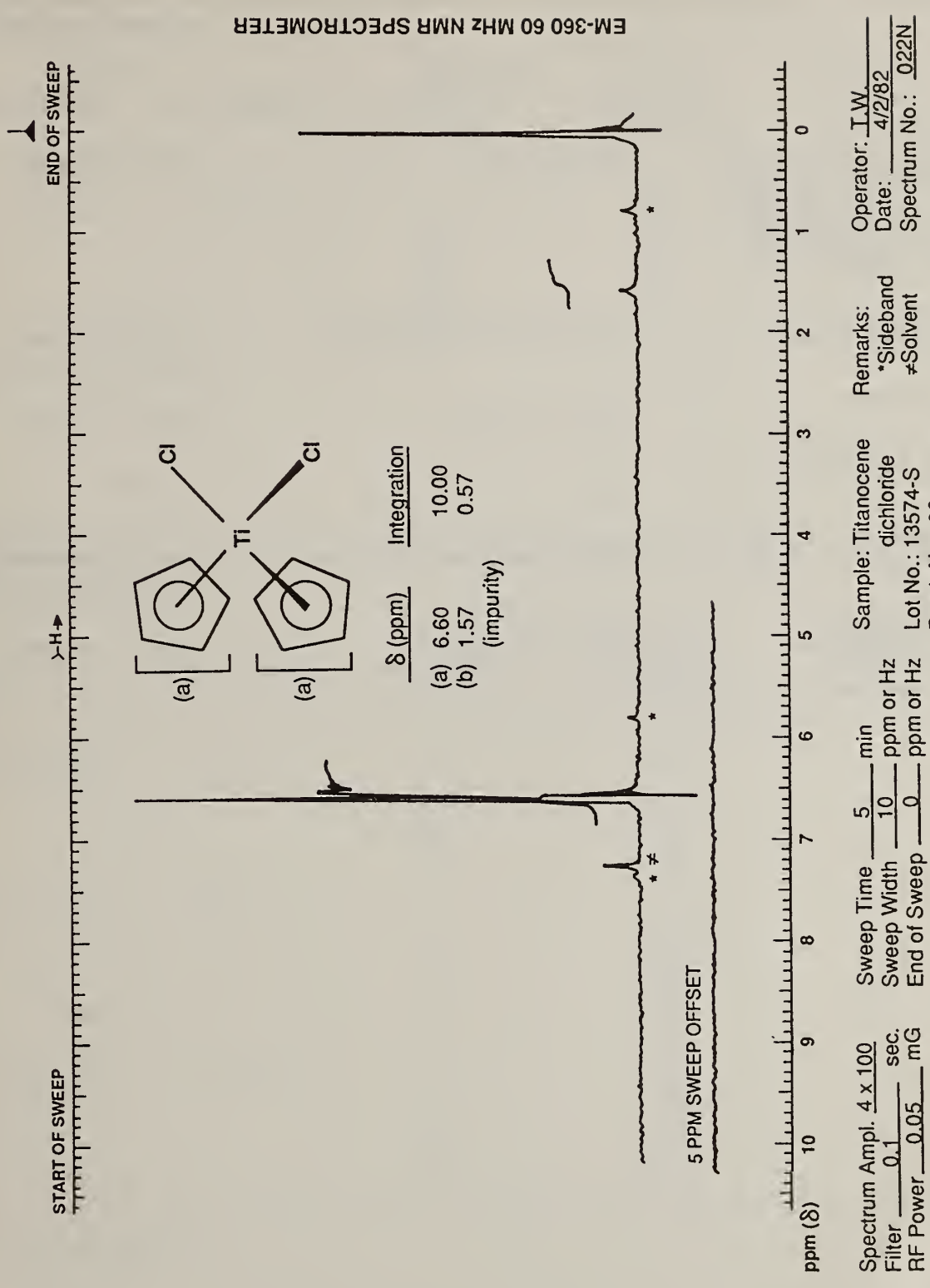

Figure G2
Nuclear Magnetic Resonance Spectrum of Titanocene Dichloride

TABLE G1
Preparation and Storage of Dose Formulations in the Gavage Studies of Titanocene Dichloride

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
Titanocene dichloride was mixed with Mazola® corn oil and homogenized for one minute at low speed with a Polytron blender. Dose formulations were stirred continuously during sampling to maintain homogeneity.	Same as 14-day studies	Same as 14-day studies
Concentration		
0, 12.5, 25.0, 50.0, 100.0, and 200.0 mg/mL	0, 1.6, 3.1, 6.2, 12.5, and 25.0 mg/mL	0, 5.0, and 10.0 mg/mL
Storage Conditions		
0 ± 5° C	Same as 14-day studies	Same as 14-day studies
Maximum Storage Time		
2 weeks	2 weeks	2 weeks
Study Laboratory		
EG&G Mason Research Institute, Worcester, MA	Same as 14-day studies	Same as 14-day studies
Referee Laboratory		
Midwest Research Institute, Kansas City, MO	Same as 14-day studies	Same as 14-day studies

TABLE G2
Results of Analysis of Dose Formulations for Rats in the 14-Day Gavage Studies of Titanocene Dichloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Percent Difference from Target
1 July 1981	2 July 1981	6.25	6.40	+2
		12.5	13.35	+7
		25.0	26.3	+5
		50.0	54.9	+10
		100.0	111.4	+11
		200.0	216.6	+8

[a] Results of duplicate analyses

TABLE G3
Results of Analysis of Dose Formulations for Rats in the 13-Week Gavage Studies of Titanocene Dichloride

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Percent Difference from Target
6 January 1982	7 January 1982	0.8	0.74	-7
		1.6	1.54	-4
		3.1	3.17	+2
		6.2	6.30	+2
		12.5	12.50	0
		25.0	25.80	+3
17 March 1982	19 March 1982	0.8	0.82	+3
		1.6	1.55	-3
		3.1	2.94	-5
		6.2	6.68	+8
		12.5	12.74	+2
		25.0	26.51	+6
28 April 1982	29 April 1982	0.8	0.88	+10
		1.6	1.60	0
		3.1	3.26	+5
		6.2	6.44	+4
		12.5	12.76	+2
		25.0	26.98	+8

[a] Results of duplicate analyses.

TABLE G4
Results of Analysis of Dose Formulations for Rats in the 2-Year Gavage Studies of Titanocene Dichloride

Date Prepared	Date Analyzed	Target Concentration[a]	Determined Concentration[b]	Percent Difference from Target
		(mg/mL)	(mg/mL)	
8 February 1983	8 February 1983	5.0	4.81	-4
		10.0	9.92	-1
8 February 1983[c]	15 February 1983	5.0	5.22	+4
		10.0	10.41	+4
24 March 1983	25 March 1983	5.0	4.89	-2
		10.0	10.52	+5
19 May 1983	23 May 1983	5.0	4.88	-3
		10.0	9.97	0
		(mg/g)	(mg/g)	
11 August 1983	12 August 1983	5.44	5.47	+1
		10.86	10.92	+1
11 August 1983[c]	18 August 1983	5.44	5.16	-5
		10.86	10.64	-2
28 September 1983	3 October 1983	5.44	5.64	+4
		10.86	10.88	0
8 December 1983	12 December 1983	5.44	5.34	-2
		10.86	10.90	0
19 January 1984	20 January 1984	5.44	6.08[d]	+12
		10.86	11.27	+4
23 January 1984[e]	23 January 1984	5.44	5.40	-1
		10.86	11.05	+2
23 January 1984[c]	6 February 1984	5.44	5.51	+1
		10.86	11.56	+6
22 March 1984	23 March 1984	5.44	5.57	+3
		10.86	10.95	+1
17 May 1984	21 May 1984	5.44	5.57	+3
		10.86	11.44	+5
28 June 1984	29 June 1984	5.44	5.68	+4
		10.86	11.09	+2
28 June 1984[c]	11 July 1984	5.44	5.87	+8
		10.86	10.96	+1
15 August 1984	16 August 1984	5.44	5.50	+1
		10.86	10.78	-1

TABLE G4
Results of Analysis of Dose Formulations for Rats in the 2-Year Gavage Studies of Titanocene Dichloride (continued)

Date Prepared	Date Analyzed	Target Concentration	Determined Concentration	Percent Difference from Target
		(mg/g)	(mg/g)	
3 October 1984	4 October 1984	5.44	5.51	+1
		10.86	11.56	+7
21 November 1984	26 November 1984	5.44	5.60	+3
		10.86	11.49	+6
16 January 1985	17 January 1985	5.44	5.39	-1
		10.86	11.38	+5
16 January 1985[c]	28 January 1985	5.44	5.54	+2
		10.86	11.02	+2

[a] Target and determined concentrations were expressed in terms of weight of test compound/total volume of formulation at the start of the studies and were expressed as weight/total weight after June 1983.
[b] Results of duplicate analyses.
[c] Animal room samples
[d] Out of specifications; remixed on 23 January 1984.
[e] Remix

TABLE G5
Results of Referee Analysis of Dose Formulations in the Gavage Studies of Titanocene Dichloride

Date Mixed	Target Concentration	Determined Concentration (mg/mL)	
		Study Laboratory[a]	Referee Laboratory[b]
13-Week			
01/06/82	1.6 mg/mL	1.54 mg/mL	1.43 mg/mL
2-Year			
02/08/83	5.0 mg/mL	4.81 mg/mL	4.97 mg/mL
08/11/83	10.86 mg/g	10.92 mg/g	10.56 mg/g
03/22/84	5.44 mg/g	5.57 mg/g	5.99 mg/g
10/03/84	10.86 mg/g	11.56 mg/g	9.90 mg/g
01/16/85	10.86 mg/g	11.38 mg/g	10.10 mg/g

[a] Results of duplicate analyses
[b] Results of triplicate analyses

APPENDIX H
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE H1
Ingredients of NIH-07 Rat and Mouse Ration[a]

Ingredients[b]	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE H2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 μg	
Pyroxidine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE H3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.43 ± 0.94	21.0–24.5	25
Crude fat (% by weight)	5.28 ± 0.66	4.2–6.4	25
Crude fiber (% by weight)	3.56 ± 0.32	2.9–4.5	25
Ash (% by weight)	6.65 ± 0.28	5.96–7.27	25
Amino Acids (% of total diet)			
Arginine	1.320 ± 0.072	1.210–1.390	5
Cystine	0.319 ± 0.088	0.218–0.400	5
Glycine	1.146 ± 0.063	1.060–1.210	5
Histidine	0.571 ± 0.026	0.531–0.603	5
Isoleucine	0.914 ± 0.030	0.881–0.944	5
Leucine	1.946 ± 0.056	1.850–1.990	5
Lysine	1.280 ± 0.067	1.200–1.370	5
Methionine	0.436 ± 0.165	0.306–0.699	5
Phenylalanine	0.938 ± 0.158	0.665–1.050	5
Threonine	0.855 ± 0.035	0.824–0.898	5
Tryptophane	0.277 ± 0.221	0.156–0.671	5
Tyrosine	0.618 ± 0.086	0.564–0.769	5
Valine	1.108 ± 0.043	1.050–1.170	5
Essential Fatty Acids (% of total diet)			
Linoleic	2.290 ± 0.313	1.830–2.520	5
Linolenic	0.258 ± 0.040	0.210–0.308	5
Vitamins			
Vitamin A (IU/kg)	11,488 ± 4,665	4,200–22,000	25
Vitamin D (IU/kg)	4,450 ± 1,382	3,000–6,300	4
α–Tocopherol (ppm)	43.58 ± 6.92	31.1–48.0	5
Thiamine (ppm)	20.12 ± 5.09	12.0–37.0	25
Riboflavin (ppm)	7.60 ± 0.85	6.10–8.20	5
Niacin (ppm)	97.80 ± 31.68	65.0–150.0	5
Pantothenic acid (ppm)	30.06 ± 4.31	23.0–34.0	5
Pyridoxine (ppm)	7.68 ± 1.31	5.60–8.80	5
Folic acid (ppm)	2.62 ± 0.89	1.80–3.70	5
Biotin (ppm)	0.254 ± 0.053	0.19–0.32	5
Vitamin B_{12} (ppb)	24.21 ± 12.66	10.6–38.0	5
Choline (ppm)	3,122 ± 416.8	2,400–3,430	5
Minerals			
Calcium (%)	1.21 ± 0.16	0.87–1.43	25
Phosphorus (%)	0.95 ± 0.06	0.84–1.10	25
Potassium (%)	0.900 ± 0.098	0.772–0.971	3
Chloride (%)	0.513 ± 0.114	0.380–0.635	5
Sodium (%)	0.323 ± 0.043	0.258–0.371	5
Magnesium (%)	0.167 ± 0.012	0.151–0.181	5
Sulfur (%)	0.304 ± 0.064	0.268–0.420	5
Iron (ppm)	410.3 ± 94.04	262.0–523.0	5
Manganese (ppm)	90.29 ± 7.15	81.70–99.40	5
Zinc (ppm)	52.78 ± 4.94	46.10–58.20	5
Copper (ppm)	10.72 ± 2.76	8.090–15.39	5
Iodine (ppm)	2.95 ± 1.05	1.52–3.82	4
Chromium (ppm)	1.85 ± 0.25	1.44–2.09	5
Cobalt (ppm)	0.681 ± 0.14	0.490–0.780	4

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration

	Mean ± Standard Deviation[a]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.55 ± 0.17	0.18–0.78	25
Cadmium (ppm)[b]	0.12 ± 0.04	0.10–0.20	25
Lead (ppm)	0.54 ± 0.21	0.24–1.00	25
Mercury (ppm)	<0.05		25
Selenium (ppm)	0.32 ± 0.06	0.21–0.46	25
Aflatoxins (ppb)	<5.0		25
Nitrate nitrogen (ppm)[c]	9.86 ± 4.84	2.50–22.0	25
Nitrite nitrogen (ppm)[c]	0.89 ± 1.40	0.10–6.10	25
BHA (ppm)[d]	<2.0		25
BHT (ppm)[d]	2.48 ± 1.26	1.00–5.00	25
Aerobic plate count (CFU/g)[e]	145,468 ± 148,238	6,200–420,000	25
Coliform (MPN/g)[f]	367 ± 683	3.00–2,400	25
E. coli (MPN/g)[g]	8.96 ± 29.39	3.00–150	25
	3.08 ± 0.28[h]	3.00–4.00	24
Total nitrosamines (ppb)[i]	5.67 ± 5.74	0.80–30.30	25
N-Nitrosodimethylamine (ppb)[i]	4.98 ± 5.77	0.50–30.00	25
N-Nitrosopyrrolidine (ppb)[i]	0.69 ± 0.71	0.30–2.70	25
Pesticides (ppm)			
α-BHC[j]	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.1		25
Estimated PCBs	<0.2		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.1		25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion[k]	0.15 ± 0.18	0.05–0.81	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Four lots were measured at 0.20 ppm; 22 February 1984, 14 March 1984, 09 May 1984, and 13 June 1984.
[c] Source of contamination: alfalfa, grains, and fish meal.
[d] Source of contamination: soy oil and fish meal
[e] CFU = colony forming unit
[f] MPN = most probable number
[g] Mean, standard deviation, and range include one large value of 150 MPN obtained in the batch milled October 17, 1984.
[h] Mean, standard deviation, and range exclude the value given in [f]
[i] All values were corrected for percent recovery.
[j] BHC = hexachlorocyclohexane or benzene hexachloride
[k] Fourteen lots contained more than 0.05 ppm.

APPENDIX I
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Upon arrival, 5 male and 5 female rats were sacrificed for the evaluation of the health status of the animals. Fifteen F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups to serve as sentinel animals. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Data from animals surviving 24 months were collected from 5 randomly selected control animals of each sex. The blood from each animal was collected and clotted, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the antibody titers. The following tests were performed:

Test	Time of Analysis
ELISA	
RCV/SDA (sialodacryoadenitis virus)	6, 12, 18, and 24 months
Mycoplasma pulmonis	24 months
Mycoplasma arthritidis	24 months
Hemagglutination Inhibition	
PVM (pneumonia virus of mice)	6, 12, 18, and 24 months
KRV (Kilham rat virus)	6, 12, 18, and 24 months
H-1 (Toolan's H-1 virus)	6, 12, 18, and 24 months
Sendai virus	6, 12, 18, and 24 months

RESULTS

The serology results for sentinel animals are presented in Table I1.

TABLE I1
Murine Virus Antibody Determinations for Rats in the 2-Year Gavage Studies of Titanocene Dichloride

Interval (months)	Number of Animals	Positive Serologic Reaction for
6	0/10	–
12	0/10	–
18	0/9	–
24	2/10	KRV

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF AUGUST 1991

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichloroethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	1,3-Dichloropropane (Telone II®)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3
336	Penicillin VK
337	Nitrofurazone

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF AUGUST 1991

TR No.	CHEMICAL
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	*a*-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3'-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
386	Tetranitromethane
387	Amphetamine Sulfate
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
393	Sodium Fluoride

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